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Motility Mutations in Flagella

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Motility Mutations In Flagella

Using Insertional Mutagenesis to Generate Mutants with Motility Defects in *Chlamydomonas reinhardtii*

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Abstract

Identification of motility mutants in flagella can directly impact cilia-related disease research as flagellar proteins are highly conserved between the green alga *Chlamydomonas reinhardtii* and humans. Insertional mutagenesis of *C. reinhardtii* allows both forward and reverse genetic analysis approaches, which could be done more efficiently if a mutant collection was available with identified insertion and deletion sites in potentially interesting genes. Transformation by electroporation allowed for 1.5 kb and 1.7 kb fragments conferring Hygromycin resistance to insert randomly into the *Chlamydomonas* genome, thus generating 35 mutants, all with defective swimming phenotypes. Restriction Enzyme Site-Directed Amplification Polymerase Chain Reaction was used to identify insert-flanking sequences and thus, insert locations. Further analysis was performed on two mutants: a flagellar protein ODA1 mutant and a Calcium ATPase mutant. Western blot analysis of the ODA1 mutant showed little to no signal of the ODA1 and Docking Complex 3 (DC3) proteins, which are involved in ODA-docking complex and outer dynein arm assembly. Exposure of the Calcium ATPase mutant to different calcium levels did not significantly affect the phenotype.

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I. Introduction

Eukaryotic cilia and flagella can be found in many different organisms. Cilia are located in many different areas in the human body, such as the trachea, fallopian tubes and the renal tube cells, among others. This widespread presence throughout the body is due to cilia's ability to perform three different functions: motility, transportation of materials and signal reception. Due to the widespread presence throughout the body, dysfunctional cilia and flagella cause many different conditions and diseases. Some examples are primary ciliary dyskinesia, polycystic kidney disease, Bardet-Biedl syndrome and a number of other ciliopathies (Ibañez-Tallon, Heintz, & Omran, 2003). Studying how mutations in genes encoding flagellar proteins occur and their effect on flagellar motility can provide insight on disease mechanisms as well as on how to cure these diseases. The biflagellated green algae *Chlamydomonas reinhardtii* can be used as a model organism where mutations can be induced and analyzed. This paper will discuss a method of generating *C. reinhardtii* mutants as well as the analysis of two specific mutants.

A. Flagellar Structure

While eukaryotic cilia and flagella may differ in function, movement and location, they are essentially identical in structure. Flagella tend to be longer than cilia, which are shorter and usually by the thousands in the body due to numerous cilia covering a cell. There are two different kinds of cilia: primary and motile. Primary cilia are immotileand mostly act as signal receptors while motile cilia are often used for transportation purposes in the human body. Despite both being motile, flagella and motile cilia differ in movement: cilia tend to move with a stiff rowing motion but with a flexible return whereas flagella

undulate continuously in a whip-like motion (Chiras, 2008). Since their inner structures are essentially identical, for this paper the two terms shall be used interchangeably.

1. Flagellar Axoneme and Microtubules

Flagella contain an axoneme, a cytoskeletal structure that gives support as well as flexibility. The axoneme is covered by a ciliary membrane that continues into the plasma membrane of the cell (Pazour, Agrin, Leszyk, & Witman, 2005). Inside the axoneme are microtubules composed of α- and β-dimers. These microtubules are arranged in a "9 +2" arrangement, which consists of 9 microtubule pairs surrounding a central microtubule pair. Each peripheral microtubule pair is composed of an A and B tubule. Figure 1 shows this internal arrangement in a diagram as well as an electron micrograph (Pazour, Agrin, Leszyk, & Witman, 2005). Both the peripheral microtubule pairs and the central pair of microtubules run the length of the axoneme. Flagella and motile cilia have a central pair, while primary cilia do not (Ibañez-Tallon, Heintz, & Omran, 2003).

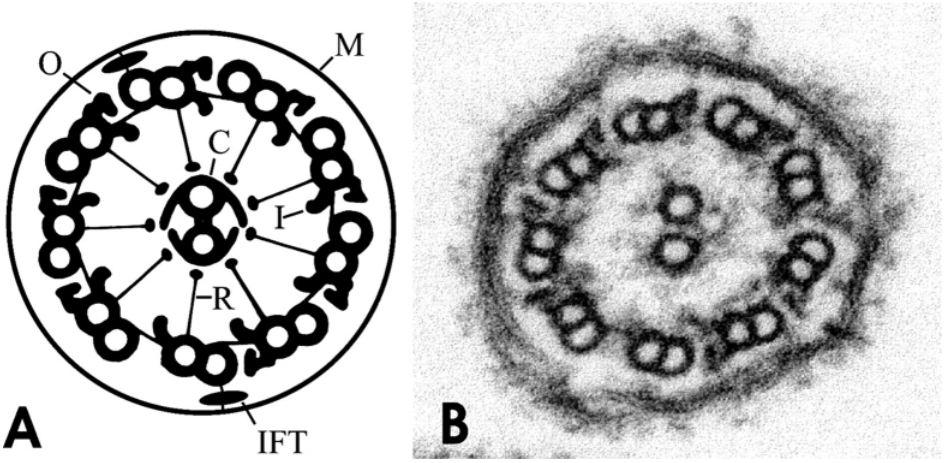

Figure 1: Flagellar structure. A diagram (A) and electron micrograph (B) both show the cross section of *C. reinhardtii* flagella. Abbreviations in (A) are [C], central microtubule pair; [I], inner dynein arm; [O], outer dynein arm; [R], radial spoke; [IFT], intraflagellar transport molecule. Image taken from Pazour, Agrin, Leszyk & Witman (2005).

2. The Dynein Arms and Radial Spokes

Other components in the axoneme include the dynein arms and the radial spokes, both illustrated in Figure 1. Regulatory signals are sent to the dynein arms through the radial spokes, signaling microtubules to slide against one another, which result in flagellar beating. The dynein arms are attached to each A-microtubule of the 9 peripheral microtubule pairs. Each arm is composed of multisubunit molecular motors that generate motion through ATP-dependent reactions. These multisubunit molecular motors are formed by polypeptide chains of different sizes: the heavy, intermediate and light chains. The heavy chains (HC) have a molecular mass of 400-500 kiloDaltons (kDa), the intermediate chains (IC) a mass of 45-110 kDa and the light chains (LC) a mass of 8-55 kDa. ATPase activity located in the heavy chain molecules provides the energy for microtubules to slide against one another (Ibañez-Tallon, Heintz, & Omran, 2003).

a) The Inner Dynein Arm

There are two dynein arms on the A-microtubules of every peripheral microtubule pair: the inner and outer dynein arms (ODA), both of which are shown in Figure 1. Both arms are composed of 30-40 different axonemal dyneins in different combinations. The inner dynein arm has several isoforms: one two-headed isoform and six single-headed isoforms. Every isoform uses different heavy, intermediate and light chains, some combinations of which are still unknown. All six single-headed isoforms associate with actin (Ibañez-Tallon, Heintz, & Omran, 2003). The single-headed isoforms consist of a single heavy chain that is associated with one actin molecule and either centrin (a Ca^{2+}-binding protein) or the p28 light chain. The double headed isoform, termed inner arm dynein I1/f, is composed of two heavy chains (1α, 1β), three intermediate chains (IC140, IC138, IC97) and five different long chains (I1/f-specific Tctex1 and Tctex2b, LC7a, LC7b, LC8) (Witman, 2009).

The heavy chains I1/f 1α and 1β are encoded by the DHC1 and DHC10 genes respectively. Both heavy chains can translocate microtubules in vitro and contribute to motility in vivo. Two of the intermediate chains, IC140 and IC138, are related to the IC1 and IC2 intermediate chains found in outer dynein arms. The C-terminal of the IC140 chain can bind to mutant axonemes lacking the inner dynein arm. The IC138 chain is a 111 kDa phosphoprotein that plays a central role in flagellar motor activity regulation. The third intermediate chain, IC97, is a 90-100 kDa polypeptide that interacts directly with α- and β-tubulin, which make up microtubules in dimerized form. Three of the light chains, LC7a, LC7b and LC8, are also found in the outer dynein arms and shall be discussed in more detail below. The remaining two light chains, I1/f-specific Tctex1 and Tctex2b, are specific to the inner dynein arm. Tctex1 seems to be more closely related to DYNLT1, a murine protein, than the LC9 chain that is part of the outer dynein arm. Tctex2b seems to play a role in the stabilization of the inner dynein arm through salt-sensitive interactions (Witman, 2009).

b) The Outer Dynein Arm

The outer dynein arm is distributed along the length of the A-microtubule at 24 nm intervals. It is responsible for producing up to four-fifths of the force required for flagellar movement as compared to the inner dynein arm, which provides the remaining force (Takada et al., 2002). The outer dynein arm structure is well characterized, as compared to the inner dynein arm. It is composed of three heavy chains (α, β and γ), two intermediate chains (IC1 and IC2) and eleven light chains (LC1-6, LC7a, LC7b, LC8-10), all of which are illustrated in a diagram in Figure 2 (Witman, 2009).

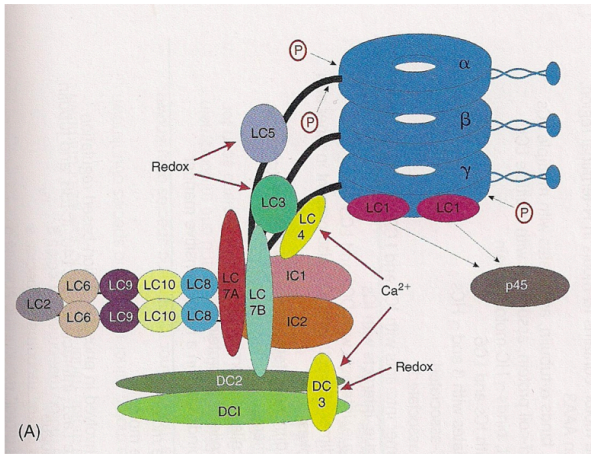

Figure 2: Diagram of the outer dynein arm structure. Image taken from Witman, 2009.

(1) The Outer Dynein Arm Heavy Chains

The outer dynein arm heavy chains have a mass of ~520 kDa and contain ~4500 residues, among which are ATP hydrolysis and ATPase sites (Pazour & Witman, 2000; Witman, 2009). One distinction of outer dynein arm heavy chains is that they directly associate with long chains, which may be involved in regulating motor function. The N-terminal region of the heavy chains have ~1800 residues, and is known to aid regulatory signal transduction, but other functions remain uncertain still. A possible feature in the N-terminal region of the γ heavy chain of outer dynein arms is the location of an ATP-insensitive microtubule-binding site (Witman, 2009).

The N-terminal region is followed by a dynein motor unit, which is made up of six AAA^+ domains in a heptameric ring along with a microtubule-binding site and a C-terminal of unknown function. Each AAA^+ domain makes up two subdomains: a helical region and an α/β structure. The helical region can detect if the terminal γ-phosphate is present using ligands. It also contains a sensor segment that undergoes nucleotide hydrolysis, which results in conformational change. The α/β structure, on the other hand, acts as a nucleotide-binding motif and is responsible for coordinating Mg^{2+} through the acidic Walker B box. It

also contains the GX_4GKT/S motif, which becomes liganded with phosphates from ATP (Witman, 2009).

(2) The Outer Dynein Arm Intermediate Chains

There are two intermediate chains present in the outer dynein arm: IC1 and IC2. Both are WD repeat proteins, which mean that each intermediate chain protein contains seven WD repeats that fold and form a β propeller structure in the C-terminal region. Each WD repeat forms one propeller blade from three out of four β strands, with the remaining β strand forming the adjacent blade. This results in a very stable structure with multiple protein-protein interaction surfaces. These chains can interact with different light chains, thus forming an IC/LC subcomplex. These interactions are essential for dynein particle assembly and stability. The intermediate chains are also very likely to be important for dynein attachment in the axoneme (Witman, 2009).

IC1 is an intermediate chain necessary for assembly of the outer dynein arm. It has been shown through cross-linking studies that that IC1 interacts with α-tubulin in situ, specifically its N-terminal region. The N-terminus is the location of a segment involved in microtubule binding. These imply that IC1 is involved in mediating the ATP-insensitive attachment of the outer dynein arm to the A-microtubule. Another possible function of IC1 is Ca^{2+} regulation of dynein function. This is due to the interaction of IC1 with a calmodulin homologue (LC4) only when Ca^{2+} is present (Witman, 2009).

IC2 is the other intermediate chain in the outer dynein arm. It is also necessary for outer dynein arm assembly. The N-terminus of this intermediate chain contains a region involved in the binding of a light chain. The C-terminus, on the other hand, contains a ~56-residue region that is predicted to form a coiled coil, which may interact with the docking complex proteins (discussed in Outer Dynein Arm Docking Complex) (Witman, 2009).

(3) The Outer Dynein Arm Light Chains

As previously mentioned, there are eleven light chains in the outer dynein arm. These eleven can be divided into two groups: those that directly associate with the heavy chain motors and those that become part of the IC/LC complex. The light chains that fall into the former group include: LC1, LC3, LC4 and LC5. The remaining chains (LC2, LC6, LC7a, LC7b, LC8, LC9 and LC10) form part of the IC/LC complex. Both the motors and the IC/LC complex are utilized in dynein assembly and direct regulation of motor activity (Witman, 2009).

c) The Outer Dynein Arm-Docking Complex

Each inner and outer dynein arm binds to a site specific for that dynein on the microtubule. Consequently, the sites must be unique from one another to ensure that only proper dynein will bind to them. Aside from the outer dynein arms and the A-microtubules, an additional factor is needed for efficient assembly and binding of the outer dynein arm onto A-microtubules. This factor has been termed the outer dynein arm-docking complex (ODA-DC). It was discovered that without the ODA-DC, outer dynein arms would not bind to the A-microtubule. This was seen in studies of *Chlamydomonas reinhardtii* mutants lacking outer dynein arms. In vivo experiments also showed that the ODA-DC could bind to the A-microtubules even in the absence of outer dynein arms (Takada, Wilkerson, Wakabayashi, Kamiya, & Witman, 2002).

The ODA-DC is composed of 3 different polypeptides in equimolar amounts: DC1, DC2 and DC3 with molecular masses of 83, 62 and 21 kDa, respectively. DC1 and DC2 are both coiled-coil proteins while DC3 is a homologue of Ca^{2+}-binding calmodulin. DC1 and DC2 are encoded at the ODA3 and ODA1 loci and are the major structural components of the docking complex. Both proteins are essential for outer dynein arm assembly (Witman, 2009). A partial docking complex, the result of peripheral microtubule pairs lacking outer

dynein arms, showed that DC1 and DC2 can be assembled without DC3 present. The opposite is also true, as DC3 cannot assemble without DC1 or DC2 (Casey, et al., 2003). Studies of mutants lacking outer dynein arms show that decreased amounts of DC1 and DC2 protein can be a cause. Much like the distribution of outer dynein arms throughout the A-microtubule, DC1 occurs at 24 nm intervals when assembled in the flagellum (Witman, 2009). This implies that the ODA-DC specifies periodicity of the outer dynein arms (Casey, et al., 2003).

DC3 is a member of the calmodulin, troponin C, essential and regulatory myosin light chains (CTER) group. It is a 21 kDa protein encoded at the ODA14 locus and a new member of the EF-hand superfamily of calcium-binding proteins (Casey, et al., 2003). It has four EF hands, which are helix-loop-helix structural domains where Ca^{2+} ions can be coordinated by ligands within the loops. The ions usually bind to the loop region, usually twelve amino acids long. EF hands usually appear in the structural domains of calcium-binding proteins such as calmodulin and troponin-C (Branden & Tooze, 1999). DC3-null mutants showed that both DC1 and DC2 proteins assemble normally, implying that DC3 is not necessary for DC1 and DC2 to integrate within the axoneme. Western blots of DC1- and DC2-null mutants axonemes showed that even if DC1 and DC2 can assemble on the axoneme without DC3, both are needed for DC3 to assemble onto the axoneme (Casey, et al., 2003). Despite its association with calcium-binding, it does not act as an outer arm Ca^{2+} sensor for Ca^{2+}-regulated outer dynein arm activity (Witman, 2009).

B. Flagellar Function and Location

As previously stated, flagella and cilia can have one of three functions: motility, transportation of liquids and objects and signal reception. Cilia and flagella provide these functions in many eukaryotes, not just humans. The structures of eukaryotic cilia and

flagella and the proteins that comprise them are highly conserved from unicellular organisms such as *Chlamydomonas* to mammals (Ibañez-Tallon, Heintz, & Omran, 2003; Pazour & Witman, 2000).

Flagellar movement follows one of two patterns: either the asymmetric (ciliary) or the symmetric (flagellar) waveform. Each type dictates the direction in which the organism will move. Asymmetric waveform swimming propels the cell forward, with the flagella leading and the cell body following behind. This type of movement is exhibited by cilia in the trachea and the oviduct. Symmetric waveform swimming has the cell swimming in reverse, with the cell body leading and the flagella undulating behind. This type of flagellar movement can be seen in mammalian sperm cells, and can be induced in *C. reinhardtii* as a photophobic response. Both waveforms are illustrated in Figure 3, which show asymmetric waveform flagellar movement in *C. reinhardtii* step-by-step on the left, and symmetric waveform flagellar movement on the right (Smith & Lefebvre, 1996).

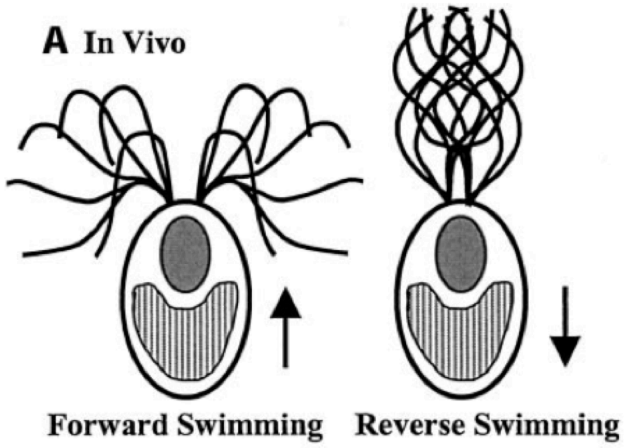

Figure 3: Flagella in *Chlamydomonas reinhardtii* have two different motility patterns: asymmetric (R) and symmetric (L) waveforms. Numbers denotes the order of movement. Image taken from Smith E. F., 2002.

Cilia are often used in the body as a means of transporting material. The forward-rowing motion and flexible return of cilia allow for transportation of materials in one direction. They can be found on epithelial cells, specifically simple columnar epithelium and

pseudostratified columnar epithelium, the latter lining the respiratory passages. Cilia in the respiratory tracts are responsible for moving mucus that has trapped dust particles and microorganisms away from the lungs. This is necessary to prevent respiratory infections (Shier, Butler, & Lewis, 2006). Cilia also line the oviduct in the female respiratory system. They help the ovum coming from the ovary during ovulation into the oviduct and guide it into the uterus. When a sperm is present in the uterus, it must swim against the downward motion of the cilia, which is necessary to bring the ovum into the uterus. Cilia can also be found on the ependymal cells of the brain. Ciliated ependymal cells line ventricles and produce cerebrospinal fluid (CSF), which cushions and bathes the brain and spinal cord. This is achieved by the ciliated ependymal cells, which ensure that CSF flows through the ventricles of the brain and around the brain and spinal cord (Sherwood, 2001).

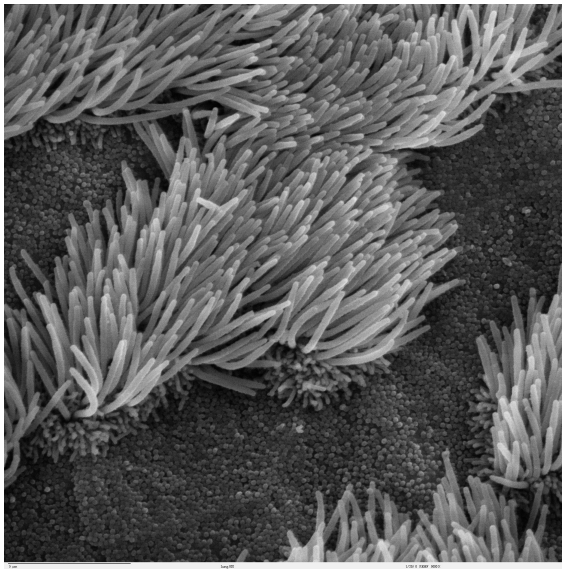

Figure 4: Scanning electron microscope image of lung trachea epithelium from a mammal. Cilia are the long projections, the rest being microvilli on a non-ciliated surface. Image taken from Dartmouth Electron Microscope Facility website.

Aside from motility and transportation, cilia, especially primary cilia, can function as signal receptors. The endothelium that covers the back of the cornea in the eye has monocilia, which may have a sensory function necessary for maintenance of corneal integrity. Vertebrate photoreceptor cells in the eye also have cilia. They are polarized

sensory neurons made up of a photosensitive outer segment bridged to an inner segment by a primary cilium (Ibañez-Tallon, Heintz, & Omran, 2003). As more research is completed in this area, more is being discovered about the effects of dysfunctional cilia in mammals. Studies on mouse mutants show that cilia play a key role in several paracrine signaling cascade transductions. These signaling events and pathway play a crucial role in establishing cell polarity and axis of symmetry as well as cell specification and differentiation, among others. Yet another area of the body where cilia can be found is the kidneys. Studies of polycystic kidney disease (a ciliopathy) indicate the presence of a Ca^{2+} channel localized in the primary cilium of renal epithelial cells. This channel is formed by two novel proteins that not only interact with each other but also could function as mechanosensors of extracellular fluid flow signaling to the cell interior through Ca^{2+} flux regulation. This implies that primary cilia in renal epithelium could act as environmental sensors for cell growth and differentiation regulation. (Badano, Mitsuma, Beales, & Katsanis, 2006).

The functions and locations of cilia are numerous, especially in the human body. Much is still not known about ciliary function in the renal epithelium cells. It is also unknown if all locations of cilia in the body have been discovered. Overall, the study of cilia and flagella is relatively new, and discoveries are being made that indicate cilia and flagella to be organelles of extreme usefulness and importance to the body.

C. Importance of Ciliary and Flagellar Protein Research

As mentioned above, eukaryotic cilia and flagella structures and proteins are highly conserved. This allows for research on a unicellular organism such as *Chlamydomonas*, whose flagella can be easily extracted and analyzed genetically and biochemically. Results from experiments on an organism such as this can directly impact research on a group of

diseases caused by dysfunctional or non-functional cilia and flagella, collectively known as ciliopathies. Many *Chlamydomonas* flagellar proteins have homologues in many different species, notably human proteins. *Chlamydomonas* flagellar proteins in the outer dynein arm can have between 40-92% identity with human proteins (Pazour & Witman, 2000). This means that mutations caused and found in *Chlamydomonas* can be models for ciliopathy disease mechanisms as well as other dysfunctional cilia-caused conditions. Due to its widespread presence in the human body, ciliary dysfunction can cause a variety of diseases and conditions throughout the body. One example of a ciliopathy is primary ciliary dyskinesia (PCD). Patients with PCD experience recurring infections of the upper and lower respiratory tracts because the cilia lining trachea are unable to transpoet mucus away from the lungs. This is because motile cilia in PCD are either immotile, dysmotile or absent (Chiras, 2008). Other examples of cilia-related diseases and conditions are: primary ciliary dyskinesia (PCD), male infertility, female subfertility, polycystic kidney disease (PKD), nephronophthisis and polycystic liver disease (Ibañez-Tallon, Heintz, & Omran, 2003).

It is important to note that a person with a ciliopathy can experience associated diseases and conditions. An example of this is when a patient with PCD also has situs inversus, which is the reversed placement of vital organs, i.e. the heart is located on the right side of the body instead of the left. The occurrence of situs inversus with PCD is due to immotile or dysfunctional nodal monocilia, which cover the ventral surface of the embryonic node in mammals, being unable to initiate nodal flow. Nodal flow is an initiating event for the determination of the left-right patterning of an embryo. Nodal monocilia rotate in a clockwise fashion, which generates a leftward or "nodal" flow of fluid surrounding an embryo. When nodal flow is impaired, left-right patterning of the embryo is affected, and situs inversus occurs. (Gilbert, 2010; Ibañez-Tallon, Heintz, & Omran, 2003). PCD patients can also experience hearing loss due to the dysfunctional cilia in the middle ear. These

examples demonstrate the multi-organ effect dysfunctional cilia can have on the human body. Studying flagellar mutations and understanding their resulting effects can hold the key to a possible cure for many ciliopathies (Ibañez-Tallon, Heintz, & Omran, 2003).

D. *Chlamydomonas* as a Model Organism for Flagellar Research

Chlamydomonas is a genus of unicellular biflagellated green alga. Each cell has two flagella of equal length located at the anterior end emerging from basal bodies. Both flagella emerge from the cell body at a close distance. Near the flagella bases are contractile vacuoles and the Golgi apparatus. The *Chlamydomonas* cell has a nucleus, mitochondria, as well as lysosomes, peroxisomes and glyoxysomes. The cells tend to be oval in shape, with a glycoprotein-rich cell wall and a single chloroplast, where an eyespot is anteriorly located. It can be found worldwide in a diversity of habitats. Species have been isolated from freshwater ponds, sewage ponds, forests, deserts, damp walls and even from mattress dust in the Netherlands. Of all the different *Chlamydomonas* species, the *C. reinhardtii* species is the species of choice for genetic studies (Harris, 2009).

Chlamydomonas has proven to be an excellent biochemical and genetic model system. As previously mentioned, many *Chlamydomonas* flagellar proteins have homologues in many different species, notably human proteins, and can be 40-92% identical. Human orthologs of most *Chlamydomonas* ODA subunits are already present in genome data banks. This means that *Chlamydomonas* is an ideal model organism on which to study flagellar structure and mechanism, as research done on *Chlamydomonas* can have a direct impact on ciliopathy research as well as other areas of human health and biology. In addition to this, one can use biochemical, cell biological and genetic approaches to analysis. The *Chlamydomonas* genome is small, comparable in size to other model organisms such as the 157 million base pair genome of *Arabidopsis thaliana* and the 100 million base pair genome

of *Caenorhabditis elegans*. It can be cultured in large amounts at low cost, making transformation and flagella isolation easy. Methods have already been developed to purify large amounts of axonemal dyneins and kinesins, which can be used for protein chemistry studies. *Chlamydomonas* is haploid but it can be induced to form diploids, making isolation and analysis of recessive mutations straightforward. It also makes it possible for testing if two mutations are allelic. Mutation analysis can be done with either tetrad genetics or restriction fragment length polymorphism (RFLP) maps of the chromosome. All these reasons make *Chlamydomonas* an excellent model organism, especially for flagellar research (Pazour & Witman, 2000). For the project discussed in this paper, the species *Chlamydomonas reinhardtii (C. reinhardtii)* was used for insertional mutagenesis.

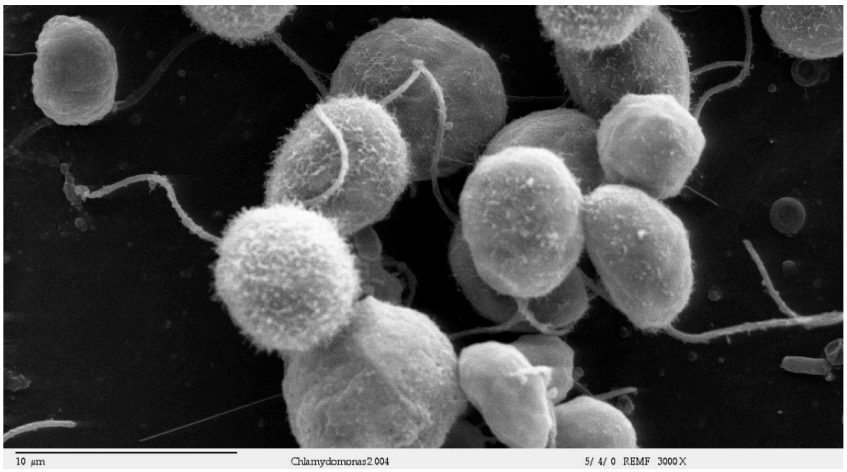

Figure 5: A scanning electron microscope image of Chlamydomonas reinhardtii cells. Image taken from Smith & Lefebvre, 1996.

E. Using Insertional Mutagenesis to Generate Mutants in *C. reinhardtii*

Given all the previous reasons for using *Chlamydomonas* as a model organism to study flagellar mutations and its importance, there is a need for a catalogue of a collection of mutants with disrupted gene sites and their resulting phenotypes. This catalogue would greatly expedite forward and reverse genetic analyses done on *Chlamydomonas*. This paper

will discuss the process of generating mutants using insertional mutagenesis and the catalogue of resulting mutants, as well as further genetic analysis of selected mutants.

Mutations can be induced in *Chlamydomonas* through insertional mutagenesis, which uses the process of transformation to incorporate a selectable marker (such as drug resistance) randomly into the *Chlamydomonas* genome (Pazour & Witman, 2000). *Chlamydomonas* cells that take up the fragment can be selected on plated selective media and placed into 96-well plates for phenotype screening. DNA is extracted from cells with the desired phenotype. Restriction Site-Directed Amplification Polymerase Chain Reaction (RESDA-PCR) is performed on the extracted DNA, which can amplify the sequences flanking the inserted fragment. RESDA-PCR uses specific primers of the marker DNA along with degenerate primers that will anneal to restriction site sequences highly and randomly distributed throughout the genome, thus amplifying the DNA sequence adjacent to the marker (Gonzalez-Ballester, de Montaigu, Galvan, & Fernandez, 2005). Identifying the sequences flanking the inserted marker allows for determining the insert location of the marker as well as any deletions or disruptions that may have occurred in the genome. The BLAST services offered by the National Center for Biotechnology Information (NCBI) and the U.S. Department of Energy's Joint Genome Institute *Chlamydomonas reinhardtii* genome portal can be used to identify insert locations as well as possible homologues. As mutants are generated, one can decide to perform genetic analysis on a mutant with an interesting phenotype and/or disruption in a gene of interest.

This mutagenesis project utilized the processes described above to generate 35 *C. reinhardtii* mutants, all with various flagellar motility defects. Insertional mutagenesis was achieved using 1.5 kb and 1.7 kb pHyg3 fragments for transformation by electroporation. Insert locations of both fragments were determined through RESDA-PCR that was performed on all mutants. One mutant, DR10-3c 4a9, showed that the 1.5 kb pHyg3

fragment had inserted into the 5th exon of the *ODA1* gene, which encodes the ODA-DC protein DC2. A Western blot was used to determine what effect the insertion had on the gene and its protein product as well as to see if any other docking complex proteins were affected. The blot showed significantly decreased DC2 protein amount as well as DC3 (the third ODA-DC protein) compared to wild-type amounts. Another mutant generated through the same methods previously describe was also analyzed. JB BG8, a mutant in the gene encoding a known flagellar Ca^{2+} ATPase, was placed in environments with different calcium conditions and observed for effect on the phenotype, which was twitchy swimming. The cells were incubated in the different calcium conditions overnight, but this did not seem to rescue the phenotype, so movies were taken of the cells so swimming velocities could be measured. It was observed that mutant Ca^{2+} ATPase cells in no calcium and calcium conditions had the slowest overall swimming velocities.

II. Methods

The methods section is an important part of every scientific paper, as it allows for other researchers to understand the reason for the methods chosen as well as replicate the experiments and procedures used. It also serves to educate a reader unfamiliar with the area of research on how the results were obtained. The following section will elaborate on the methods used for this mutagenesis project of *C. reinhardtii*.

A. Plasmid Purification

The starting point of a project using insertional mutagenesis to generate mutants is the fragment used for transformation and how it was constructed. This section will elaborate on the methods used to construct the 1.5 kb and 1.7 kb pHyg3 fragments.

1. Lysogeny Broth Media & Growth Conditions

Lysogeny broth (LB) media with ampicillin added was used for inoculation. One liter of LB media was prepared with 10 grams of Bacto tryptone and 5 grams each of yeast extract and sodium chloride in a final volume of 1 L. 10 µL of 100 mg/mL Ampicillin was then added to 10 mL of LB media, which was distributed four sterile tubes, resulting in 2.5 mL of LB + 10 µg/mL Amp per tube.

2. Inoculation & Plasmid Purification

Sterile toothpicks were used to pick single *E. coli* colonies containing pHyg3 plasmid from a plate. Each toothpick was used to inoculate a 2.5 mL LB + Ampicillin tube. Cells were cultured overnight and harvested by centrifuging at 8000 rpm for 3 minutes, after which the supernatant was aspirated. Qiagen mini/maxi prep kits were used to purify plasmid DNA.

3. Plasmid Digest

After extraction of pHyg3 plasmid DNA from E. coli, the plasmids were cut with HindIII and BamHI restriction enzymes to generate the 1.7 kb and 1.5 kb fragments.

a) 1.7 kb Fragment

30 µg of the purified plasmid DNA was digested with 10 µL of HindIII restriction enzyme in a total volume of 250 µL. The digest incubated at 37 °C for 5 hours. The resulting plasmid should be 1.7 kb in size.

b) 1.5 kb Fragment

The 1.5 kb fragment was prepared similarly to the 1.7 kb fragment. 30 µg of DNA was digested with HindIII restriction enzyme, as above. The digest was then purified with a Qiaquick PCR purification kit in preparation for digestion with BamHI. 52 µL of the HindIII-digested DNA was digested with 30 µL BamHI in a final volume of 502 µL. This incubated at 37 °C for 5 hours. The resulting plasmid should be 1.5 kb in size.

4. Plasmid Extraction by Gel Purification

In order to obtain the correct size fragments, both digests were electrophoresed on a 1% agarose gel. The correct size bands were excised and the DNA extracted using a QiaEx II agarose gel extraction kit.

B. Culturing *C. reinhardtii* Cells

The procedures below detail how *C. reinhardtii* cells are grown.

1. Minimal (M) Media

C. reinhardtii cells were cultured in bubblers with 125 mL of minimal (M) media. 2 L of M media was made for 12 bubblers. The following were added (in order) to an initial amount of 1.5 L of double distilled water (ddH_2O): 2 mL 10X trace metals, 10 mL 10% Na

Citrate $2H_2O$, 2 mL 1% $FeCl_3 6H_2O$, 2 mL 5.3% $CaCl_2 2H_2O$, 6 mL 10% $MgSO_4 7H_2O$, 6 mL 10% NH_4NO_3, 6 mL 10% KH_2PO_4 and 6 mL 10% K_2HPO_4. Double-distilled water (ddH_2O) was used to bring the solution to a final volume of 2 L.

2. Transformation by Electroporation

C. reinhardtii cells were cultured in bubblers containing M media and under constant bubbling with room air supplemented with 5% CO_2 for 2-3 days until the culture was a medium green color. They were incubated in a culture room with 14-hour light and 10-hour dark cycles. 2 x 10^8 cells were harvested by centrifugation. After aspirating the supernatant, the pellet was washed with 10 mL of TAPS + 40 mM sucrose and resuspended in 40 mL of TAPS media, which incubated in light for four hours. After the cells were centrifuged, the supernatant was aspirated, and the pellet resuspended in TAPS + 40 mM sucrose in a 1 mL final volume.

2500 ng of 1.7 kb/1.5 kb fragment DNA was added to cells in a final volume of 800 µl . This was distributed among 10 0.1cm cuvettes, which were kept on ice for 10 minutes. Cells were then electroporated with a single pulse using a BTX ECM600 electroporator with the following settings:

Low voltage mode – 500V capacitance and resistance

Voltage set at approximately 200V

Resistance set at 13 ohms

Capacitance set at 1000 µF

Each cuvette was incubated after electroporation for 15 minutes. Cells were resuspended with 1 mL TAPS + 40 mM sucrose and added to a 15 mL conical tube containing 9 mL TAPS + 40 mM sucrose. All conicals were left on a rocker overnight at room temperature with very gentle rocking in dim light. Cells were centrifuged the next day and the supernatant aspirated. Remaining supernatant was used to resuspend the pelleted cells.

These cells were plated on 1.5% TAPS + hygromycin plates. Plates were parafilmed and grown in light until colonies were large enough to pick.

C. Mutant Screening

Colonies from transformation plates were picked using sterile toothpicks and placed into 96-well plates. The plates were grown in light until wells were a medium green (approximately 2-3 days). Cells that swam abnormally (spinning, shaky, swam slower than normal, unable to swim straight, non-moving) were transferred to a 24-well plate to confirm phenotype.

D. DNA Isolation

Mutant strains were cultured in bubblers until achieving a medium green color. Cells were centrifuged, resulting in 200-400 µL of pellet. The pellet was resuspended in a mixture of 20 mM Tris buffer at pH 7.5, 20 mM EDTA, 5% SDS and 1 mg/mL Proteinase K and incubated at 50 °C for 12-16 hours. 100 µL of 7.5M Ammonium acetate at pH 7.5 and 500 µL of TE saturated 50% phenol/50% chloroform were added and mixed by inversion before centrifugation at 10K for five minutes. Polysaccharides were removed by adding 1/7 volumes of 5M NaCl (approximately 100 µL) and 0.1 volumes of 10% CTAB in 0.7M NaCl solution (approximately 70 µL) to the supernatant and mixing well by inversion. 700 µL of 24:1 chloroform-isoamyl alcohol solution was added afterwards. Cells were centrifuged again at 10k for two minutes. 1 mL 100% ethanol was added to the supernatant and mixed by inversion before centrifugation at 10K for five minutes. After aspirating the supernatant, the pellet was washed with 1 mL 80% ethanol and centrifuged at 10k for five minutes. After aspiration of the supernatant, the pellet was air-dried until ethanol odor dissipated. The pellet was resuspended in 100 µL elution buffer from Qiagen.

E. RESDA-PCR

This protocol was adapted from the methods described by Ballester et al. (2005) and adjusted for pHyg3-generated mutants. A 96-well plate contained up to 11 mutant strains, with one column for wild-type *C. reinhardtii* genomic DNA. Each well contained 1 µL of DNA in 50ng/µL concentration and a mix of either an upstream or downstream primer (UP2 or DP4), a degenerate primer (Alu, Pst, Sac and Taq), DNA polymerase, buffer, dNTP, DMSO, $MgCl_2$ and sterile double distilled water. Each well consisted of different upstream/downstream primers and degenerate primers coupled with a different mutant strain. This primary reaction was diluted and used as a template for a secondary reaction with nested PCR primers (UP1 or DP3) (Gonzalez-Ballester et al., 2005; Matsuo et al., 2008).

All RESDA products were electrophoresed using a 300 mL 1% agarose gel. Selection of mutant strains to gel purify their RESDA products was determined by the presence of clear single bands 500 bp – 2 kb in length. Remaining RESDA products of the selected strains were placed in a 100 mL 1% agarose gel. All bands were excised and gel purified using the the QiaEx II agarose gel extraction kit.

F. Western blotting

15 mL of *C. reinhardtii* cells in culture were centrifuged. After supernatant aspiration, the pelleted cells were suspended in 0.25mL of 5x DNS + PMSF and of 1 mM PMSF. Cells were incubated in a 65 °C water bath for ten minutes. DNA was sheared with a 26 gauge needle. 15 µL of cell sample were prepared for each gel well, which incubated in an 80 °C water bath for another 10 minutes before being loaded into the gel. 10µL of marker was loaded along with the 15 µL of sample for every lane. The gel was electrophoresed with SDS Page running buffer at 100V. SDS Page running buffer was made by mixing 100 mL of 10x TGRB, 10 mL of 10% Biorad SDS in a final volume of 1 L. After electrophoresis, the gel

incubated in Western Transfer Buffer (made of 200 mL of 10x TGRB, 200 mL of methanol and 1 mL of 10% Biorad SDS in a final volume of 1 L) on a shaker. The PVDF blot was immersed in methanol and placed in the container with the gel. The membrane was placed under the gel and left to incubate on the shaker for 30 minutes. Proper assembly of the blot apparatus is shown in Figure 6.

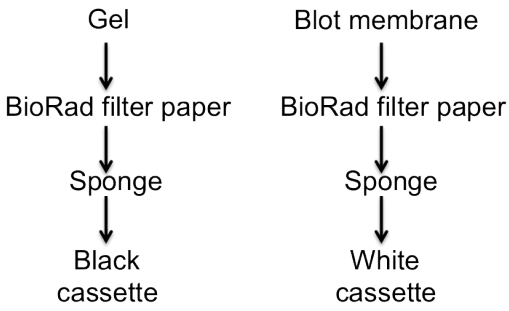

Figure 6: Proper assembly of the blot apparatus. The cassette will close, resulting in the gel and membrane on top of each other.

The transfer was run in Western Transfer Buffer at 28V on a stir plate for fifteen minutes in a cold room, after which the voltage was increased to 82V and left to transfer for 45 minutes.

Block was made with 10g of dry milk, 20 mL of 10x TBST, 4 mL of ½ strength Fish Skin Gelatin and 180 mL ddH_2O. After the transfer, the membrane was dried in the 50 °C incubator for approximately two minutes. The membrane was immersed in methanol before rinsing with ddH_2O and then with block, which was done to ensure all methanol had been rinsed off. The membrane incubated in block on a rocker for thirty minutes at room temperature. Afterwards, the membrane was placed in block diluted with primary antibody and incubated overnight on a rocker at room temperature. The next day, the membrane was washed in block on a rocker at room temperature four times over a 32-minute period (washed every 8 minutes). After the four washes, the membrane was incubated in block diluted with the desired secondary antibody (1:5000 antibody:block) for an hour on a rocker at room temperature. Afterwards, the membrane was washed four times over a 32-

minute period (same wash steps as previously mentioned). The membrane was washed with 1X TBST to remove the block. Equal amounts of reagent A and B from the KPL LumiGLO kit (warmed to approximately room temperature) were mixed (400 µL of the LumiGLO solution for every blot). Extra TBST was wicked off the membrane and was placed in the LumiGLO solution for a minute before excess solution was wicked and then placed on saran wrap. Blots were then ready for exposure.

G. Calcium Exposure and Regulation

C. reinhardtii cells (both wild-type and mutant) were cultured in bubblers until medium dark green. Observations about phenotype of each strain were made with a Nikon differential interference contrast (DIC) microscope with high NA oil condenser. 10 mL of each strain for each condition (high calcium, no calcium, M media) were centrifuged at 2000 rpm for 3 minutes. After the supernatant was aspirated, the pelleted cells were resuspended in 10 mL of the assigned condition. Cells were centrifuged again at 2000 rpm for 3 minutes. Pelleted cells were resuspended in 5 mL of the assigned condition. Cells were incubated on a light table overnight.

H. Measuring Swimming Velocities

Movies of wild-type and mutant cells in different calcium conditions were recorded using Nikon's NIS-Elements platform. ImageJ plugin "ND to Image6D" was then used to convert the movies into a stack of .tiff files. Individual cell paths were made visible using ImageJ's Walking Average plug-in, after which the Measure tool could be used on the visible path. Movies were recorded at approximately twelve frames per second with 0.32 µm/pixel quality. These numbers were used to calculate swimming velocities in Microsoft Excel, which were plotted in a histogram.

III. Results

The main goal of this research project was to generate *C. reinhardtii* mutants with flagellar motility defects and catalogue their insertion sites, but these were not the sole results. Of equal importance are the methods used to generate these results, as this is part of the pilot mutagenesis project being done by the Witman lab.

A. Overview of Insertional Mutagenesis Methods

As previously mentioned, the species *Chlamydomonas reinhardtii* was used for insertional mutagenesis. DNA fragments used for transformation were constructed from the pHyg3 plasmid, which can confer hygromycin resistance to the transformed cells. Once colonies were selected on hygromycin plates and transferred to 96-well plates, colonies were screened for mutant phenotypes. For this project, mutant phenotypes were limited to those affecting flagellar motility. After confirmation of mutant phenotype, DNA was extracted from mutants, which was then used for RESDA-PCR. Further analyses such as Western blotting and calcium exposure were performed on mutants generated with insertion in genes of interest. Figure 7 shows these methods in pictorial form.

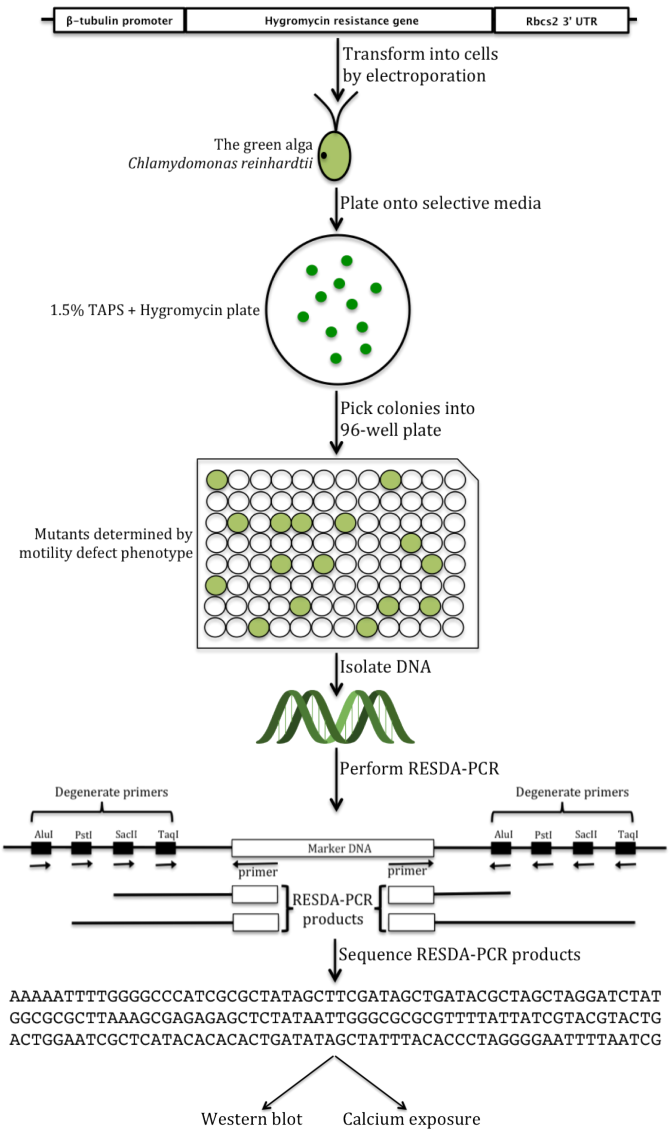

Figure 7: A pictorial representation of insertional mutagenesis methods for *C. reinhardtii*.

1. The Construction of the 1.7 kb and 1.5 kb pHyg3 Fragments

As previously mentioned, 1.5 kb and 1.7 kb fragments from the pHyg3 plasmid were used to transform *C. reinhardtii* cells by electroporation. The 1.7 kb fragment was constructed by cutting with the restriction enzyme HindIII, which cut at the two HindIII restriction sites in the pHyg3 plasmid: five base pairs before the start of the β-tubulin promoter and nineteen base pairs after the end of the 3' ribulose bisphosphate carboxylase rbcS2 UTR. The 1.5 kb fragment was constructed similarly to the 1.7 kb fragment in that it

was initially cut with HindIII as well. A second digestion was made with BamHI, which cut four base pairs after the start of the rbcS2 3' UTR. The plasmid map of pHyg3 (including the HindIII and BamHI sites) as well as the 1.7 kb and 1.5 kb maps are all shown in Figure 8.

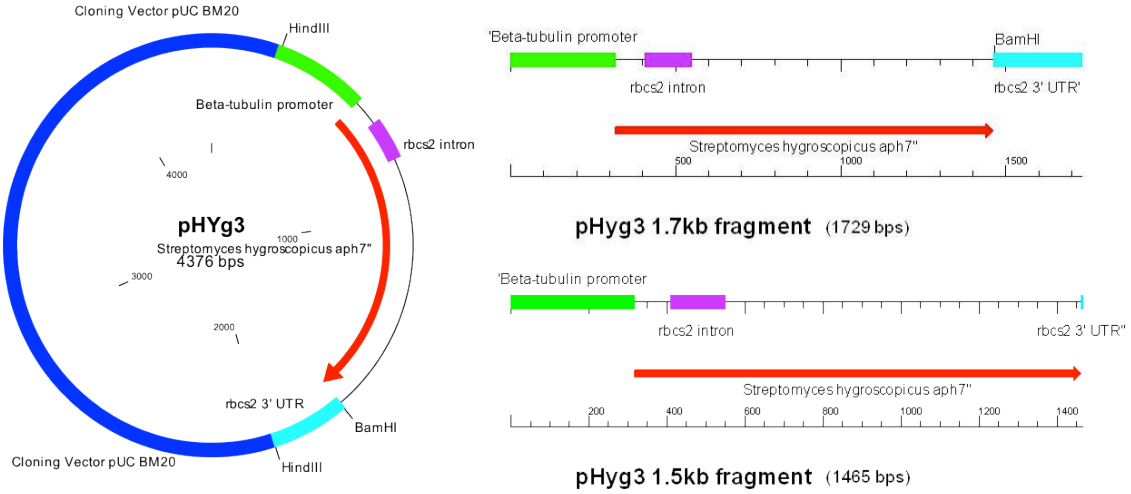

Figure 8: The 1.7 kb and 1.5 kb fragments used for insertional mutagenesis of C. reinhardtii were constructed from the pHyg3 plasmid, which confers hygromycin B resistance. Maps of all three (including HindIII and BamHI sites) are shown.

B. *C. reinhardtii* Mutants

Five transformations were performed, yielding over a thousand transformants. Out of the thousand, less than a hundred showed flagellar motility defects during phenotype screening in 96-well plates. Mutant phenotypes were confirmed by transferring cells to 24-well plates before culturing mutant strains in preparation for DNA isolation and RESDA-PCR. After RESDA-PCR was performed, the RESDA products were electrophoresed. Mutant strains whose RESDA DNA were sent for sequencing were selected based on the results of the RESDA gel. Only strains with clear, bright single bands ranging from 500 bp to 2 kb in size were sent for sequencing. The NCBI and JGI Chlamydomonas Genome Portal BLAST services were used to analyze the sequences. The results of these are seen in Table 1.

Phenotype	Batch	Mutant	Fragment	Insertion Site
Swims normally	DR10-3a	BE12	1.7 kb	-
Shaky, twitchy swimming		1a	1.7 kb	Chr. 9: C-type lectin, peptidase, trypsin-like serine and cysteine
		2a		Chr. 12: Dysferlin
		8a		-
		AC2		-
	DR2	BC11		Chr. 12
		BH7		Chr. 6: FAD/NAD-linked reductase, Pyridine nucleotide-disulphide oxidoreductase
		BC5		-
	DR10-3a	BD4		-
		BH7		-
		BD11		-
	DR10-3c	1F11	1.5 kb	-
		2F4		-
Slow swimming	DR10-3a	BE3	1.7 kb	Chr. 3
		BH10		-
Zigzag swimming, slow		3g	1.7 kb	Chr. 12: protein kinase, serine-threonine protein kinase Chr. 1: Endoplasmic reticulum protein ERp29, protein kinase, serine-threonine protein kinase Chr. 6: Histone H2A Chr. 16, 10, 17, 9, 14, 13
	DR2	AF10		-
	DR10-3a	BD11		-
	DR10-3b	AB12		Chr. 1: Pyruvate-formate lyase, Formate acetyltransferase
		AG7		-
		BB8		-
		BB10		Chr. 13: peptide chain release factor eRF/aRF subunit 1
	DR10-3c	2F4	1.5 kb	
		4A9		Chr. 16
Zigzag swimming, normal	DR10-3a	BD7	1.7 kb	-
	DR10-3b	AB9		-
		AF8		-
		BB8		-
		BC2		Chr. 12: Om/DAP/Arginine decarboxylase 2
No movement		2a	1.7 kb	Chr. 12: dysferlin
	DR10-3a	BD7		-
Palmelloid	DR2	AH11	1.7 kb	-
		BA4		Chr. 3: Serine-threonine/Tyrosine protein kinase, glycogen/starch synthase, glycosyl transferase Chr. 15: Serine-threonine/Tyrosine protein kinase Chr. 14, 1, 6, 10
		AB7		-
		BB5		-

Table 1: Different mutant strains generated by insertional mutagenesis with the corresponding phenotypes and insertion sites.

A flagellar motility mutant can have a wide variety of phenotypes because any difference in direction, speed and movement from the swimming of wild-type cell indicates a possible flagellar defect. The possible phenotypes for a flagellar motility mutant are: cells spinning, shaking, unable to swim straight, swimming slower than normal, non-moving, or any combinations thereof. One phenotype that was included in screening but is not directly related to flagellar motility is the palmelloid phenotype, which is mentioned in Table 1 and shown in **Error! Reference source not found.**. *Chlamydomonas* cells can sometimes form almelloid colonies, which are clumps of adherent, non-motile cells surrounded by a mother cell wall. Flagella mutants can sometimes result in palmelloid colonies because flagella are necessary to secrete the enzyme necessary to degrade the mother cell wall. A number of scenarios could result in a palmelloid phenotype: cells are lacking flagella, have a dysfunctional flagellar transport mechanism or the enzyme is made incorrectly (J. Brown, personal communication, April 11, 2011).

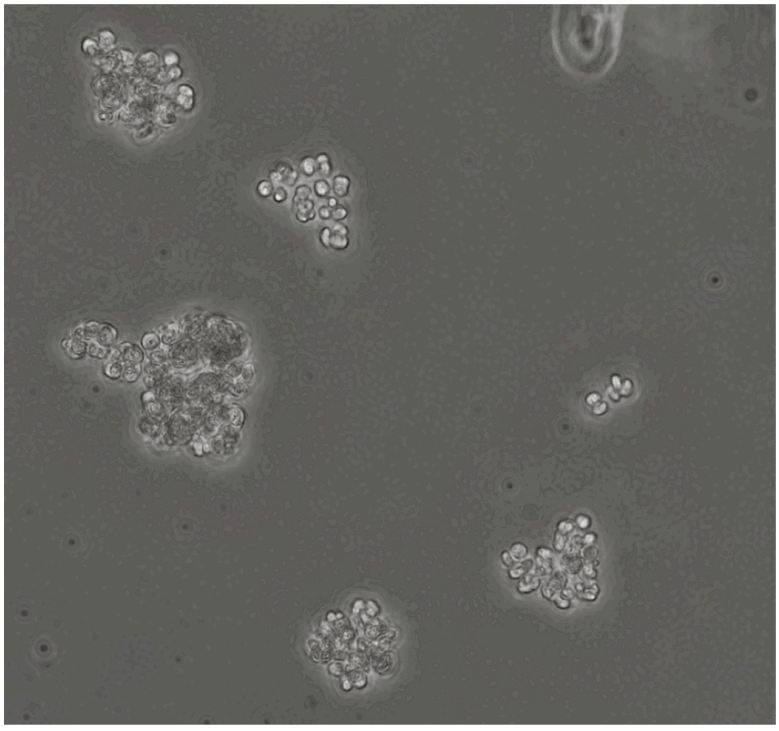

Figure 9: *C. reinhardtii* DR2AB7 is a palmelloid mutant. This phenotype is characterized by clumps of adherent, non-motile cells.

While most flanking mutant sequences did not align with any chromosomes or genes in either the NCBI or JGI Chlamydomonas Genome Portal databases, all had distinguishable phenotypes. This implied that even if the insert location was undetermined, the random integration of the hygromycin resistance fragment into the genome could have caused a flagellar motility defect. Of those mutant flanking sequences with identified insert locations, all of the identified genes were genes responsible for making various enzymes such as protein kinases and acetyltransferases, among others. All these enzymes are essential for the cell to function properly. While it is currently unknown why insertions at these particular sites cause a flagellar motility defect, the generation of mutants and cataloguing of their insertion sites is an important first step to answering those questions.

C. DR10-3c 4A9: Insertion Near ODA1

Given what is known about flagellar proteins, any mutant with an insertion in a known flagellar protein is of interest. The DR10-3c 4A9 mutant was generated using a 1.5 kb fragment, and BLAST analysis of the sequence showed that the 1.5 kb fragment had inserted into the 5th exon of the ODA1 gene locus. The ODA1 gene is responsible for the production of the ODA-DC protein DC2, a docking complex protein essential for outer dynein arm assembly (Witman, 2009). Unfortunately, only the insertion site upstream of the marker is known, as the downstream RESDA reaction did not produce a viable band for sequencing. Despite this, one can see from mapping out the flanking sequence of 4A9 where the marker most likely had inserted. The upper portion of Figure 10 shows a map of the possible insertion site of the marker and what had occurred during RESDA-PCR. The map shows where the marker-specific upstream and the Sac primers must have started amplifying towards each other, thus resulting in a flanking sequence where part of the 1.5 kb fragment inserted into the 5th exon of the ODA1 gene.

Cells of the 4A9 mutant were unable to swim straight and at a normal pace. Given that the insertion of the marker may have disrupted ODA1 gene activity, how was the ODA1 gene product, DC2, affected by the marker insertion, and if so, in what aspect? To answer this, Western blots were performed. 4A9 cells were cultured along with two wild-type strains, g1 and 137c. One blot was probed initially with βF1 ATPase, a control antibody, and then with ODA1 antibody while the second blot was probed initially with ODA1 antibody and then with DC3 antibody. DC3 is the third docking complex protein, made in the ODA14 gene locus. It interacts with DC2 and DC1 to help anchor outer dynein arms to microtubules (Witman, 2009). Both blots can be seen in Figure 10. Both blots show bands that correspond to ODA1 protein (DC2), as indicated in the image. It should be noted that the ODA1 proteins migrated at approximately 41 kDa, a lesser molecular weight than the predicted 62 kDa. The reason for this is unknown, and future analysis must be done to determine the cause.

The middle bands correspond to protein from the 4A9 mutant. DC2 protein amounts in both blots are significantly less compared to the amount of protein from wild-type cells. The other set of bands that was of interest was located below the ODA1 proteins on the right blot. These bands corresponded to the DC3 proteins. While overall in a lesser amount than DC2, significant decrease or even absence of the mutant 4a9 DC3 protein can be easily observed when comparing protein amounts with two wild-type strains. Thus, disruption of the ODA1 and ODA14 genes by marker insertion resulted in slow zig-zag swimming and decreased protein production of DC2 and DC3.

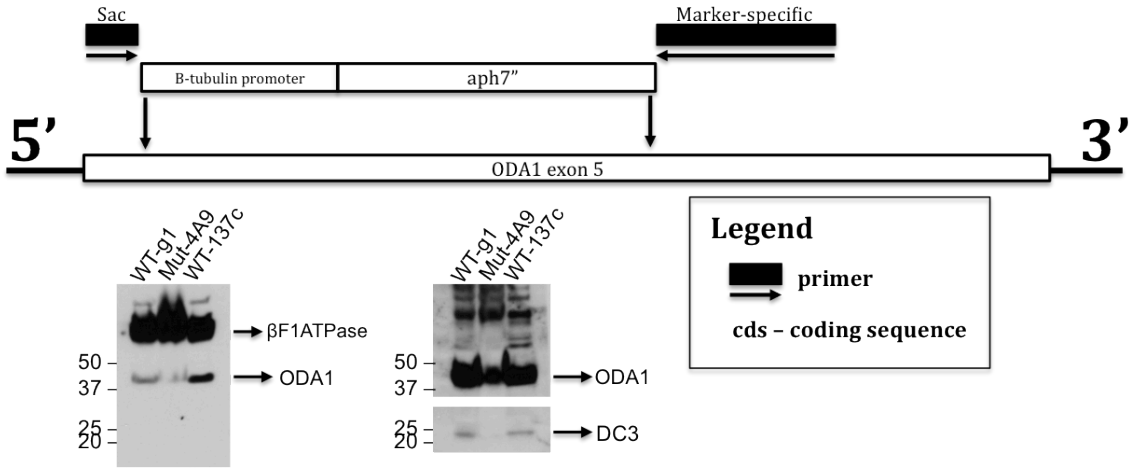

Figure 10: Further analysis done on the C. reinhardtii showed that the 1.5 kb marker inserted into the 5th exon of the ODA1 gene. The map is that of the possible insertion site of the 1.5 kb fragment and how RESDA primers amplified the sequence. Immediately below the flanking sequence map are the Western blots performed on the 4A9 protein. (L) blot was initially probed with βF1ATPase antibody then with ODA1 and (R) was initially probed with ODA1 antibody and then with DC3 antibody. Both blots were exposed for 25 minutes.

D. The Calcium ATPase Mutant BG8

Another interesting mutant analyzed was the Calcium ATPase mutant BG8. This mutant was generated using the same methods as the mutants above but by Dr. Jason Brown, the UMass advisor of this mutagenesis project. This mutant displayed a twitchy phenotype, and BLAST analysis of the sequence indicated an insert in the predicted 3' UTR of a known flagellar Ca^{2+} ATPase pump. To determine how mutant phenotype would be affected by calcium, the mutant and a wild-type strain (cc124) were placed in solutions with either 0.5 μM Ca^{2+}, 0.5 mM Ca^{2+}, 0.5mM EGTA (Ca^{2+} chelating agent) or M media (control growth media). These cultures were incubated in their respective solutions overnight. Observations with a light microscope the next day did not display any obvious phenotypic differences, so further analysis of swimming velocities was performed using the program ImageJ. Movies were recorded of each strain in each condition. Then, using the Walking Average plug-in for ImageJ, cell paths were made visible and measured using the Measure function, also in ImageJ. Velocities for each measurement were calculated according to the following formula:

$$velocity\ (^{\mu m}/_{s}) = \left(length\ (in\ pixels) \times \frac{0.32\ \mu m}{pixel}\right) \div 9.67 seconds$$

$$= \left(223.53 \times \frac{0.32\ \mu m}{pixel}\right) \div 9.67\ seconds = 7.40\ {}^{\mu m}/_{s}$$

The pixel-to-micron conversion was obtained from ImageJ, while the number of seconds was the length of all the recorded movies. Five movies of each strain in each condition were recorded. The cells in the two calcium concentrations were categorized as being in calcium because concentration did not seem to have an effect on the phenotype. The velocities of all the measured cells for every movie were averaged and plotted in Figure 11. Due to the vast differences in measurable cell numbers (only cells with visible paths were measured for path length), the graph in Figure 11 is more representative than quantitative.

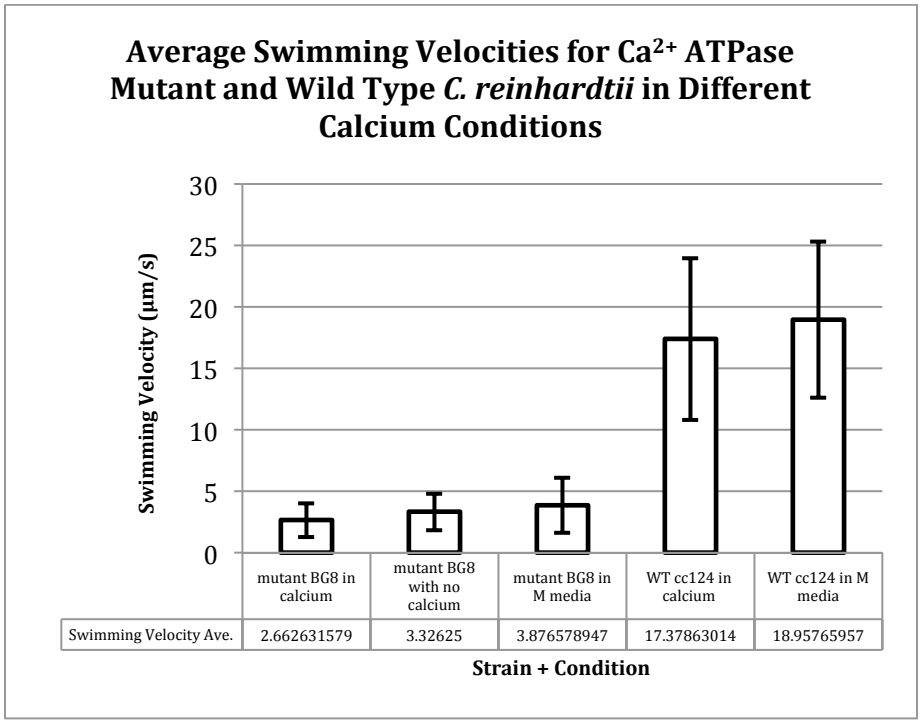

Figure 11: Average velocities of BG8 and wild type cc124 cells in different calcium conditions were measured using ImageJ.

It can be seen in Figure 11 that overall, the mutant strain swam significantly slower than the wild-type cc124 cells. Of the cc124 cells, those in calcium swam slightly slower

than those in normal growth media (M media). Of the mutant BG8 cells, it can be seen that the mutant in calcium swam the slowest overall, among the mutant cells in other conditions as well as for the entire cell culture. As expected, the fastest mutant cells were those exposed to normal growth media. The mutant cells that were not exposed to calcium did not have a significantly greater average swimming velocity than those cells exposed to calcium.

IV. Discussion

The main goal of this mutagenesis project was to generate mutants with flagellar motility defects and catalogue the insertion sites of the fragment used for mutagenesis. This meant that a project like this would be an ongoing one, as the more mutants generated with flagellar motility phenotypes and known insertion sites, the more useful the catalogue will be for forward and reverse genetic analysis. 35 mutants were generated through the method of insertional mutagenesis but not all the insertion sites were identifiable due to a number of factors. Additional genetic analysis of two other mutants provided a possible pathway of analysis for mutants generated through this method.

A. Insertional Mutagenesis Methods

The methods presented in Figure 7 are all necessary steps done in order to generate a group of mutants. Given that this mutagenesis project is still in its pilot stages, some alterations will have to be done to improve efficiency and throughput.

An area in need of improvement is the rate of successful mutagenesis by insertion. While the transformation efficiencies have been relatively high (average of 100-200 colonies per transformation plate), the number of mutants obtained from each transformation has been significantly lower. One reason could be human error in phenotype screening. Mutants for this project are determined by observing the motility of each group of cells. Some phenotypes, such as twitchy swimming, palmelloid, or non-moving, are very easy to differentiate from wild-type swimming. Other phenotypes are more difficult, such as cells unable to swim straight but do so at normal swimming speeds, or cells having one flagella slightly shorter than the other but still able to swim relatively normal. One other possibility is that flagellar motility phenotypes are coupled with other kinds of mutations. One possible example is the cell's inability to swim straight unless in light. One would be

unable to observe this phenotype because the motility phenotype will only occur during the dark cycle of the cell, therefore rendering it undetectable. Other times, mutant phenotypes may be too subtle to see. It is very possible that there are 10% more mutants than initially retrieved. This potentially increases the number of mutants obtained from each transformation.

Another possible reason for the low mutagenesis rate may be that random integration of the marker into the *C. reinhardtii* genome is too broad for the specific phenotype desired. It is possible that more targeted insertion sites are necessary for higher mutagenesis rates. Since the sequences of flagellar proteins are known, the hygromycin resistance fragment can be used to target specific flagellar proteins such as ODA-DC DC2, for example. The hygromycin fragment would have to be engineered to recombine with the target gene by incorporating sequences from the target gene into the fragment. When recombination occurs in the region of that sequence within the gene, a foreign sequence will insert into that region of the gene, thus disrupting the gene (Gilbert, 2010). Targeted disruption of a flagellar protein-producing gene may result in more flagellar motility mutants. There are currently methods under development for targeted gene disruption in *Chlamydomonas*, but the efficacies of these methods have not yet been sufficient as to be adopted by the *Chlamydomonas* research community.

One other reason for the relatively low number of mutants analyzed for this project was due to limitations by RESDA-PCR. Only strains with clear, bright, single bands in the 500 bp – 2 kb size range were gel-purified and sent for sequencing. While this diminished the probability of poor quality sequences, it also severely limited what strains could be analyzed. An example of this is the mutant DR10-3c 4A9. As mentioned above, the hygromycin fragment had inserted into the 5th exon of ODA1 gene locus. It is unknown what was located downstream of the hygromycin fragment because the downstream portion of

the RESDA-PCR did not result in any bands. This could be due to any number of things: the downstream marker-specific primer did not anneal to the marker, the degenerate downstream primers did not anneal, among others. Whatever the reason, RESDA-PCR limits the number of mutants available for genetic analysis.

B. The 1.7 kb and 1.5 kb Hygromycin Fragments

The 1.7 kb and 1.5 kb hygromycin fragments constructed from the pHyg3 plasmid are an important part of this mutagenesis project, as they integrate randomly into the *C. reinhardtii* genome through transformation by electroporation. These fragments contained the *Streptomyces hygroscopicus* aminoglycoside phosphotransferase gene *aph7"* which was used as a selectable marker. Aminoglycoside-inactivating phosphotransferases are encoded by various bacterial genes and can confer resistance to aminoglycoside antibiotics such as neomycin, gentamycin and hygromycin B, all of which *C. reinhardtii* cells are sensitive to. Hygromycin B resistance was used as a selectable marker in this mutagenesis project. Transcription of this gene is controlled by three elements: the *C. reinhardtii* β2-tubulin promoter, the *C. reinhardtii* rbcS2 intron 1, and the 3' UTR rbcS2. The β2-tubulin promoter is a strong and constitutive promoter that allows for continuous transcription of the aph7" gene. The *C. reinhardtii* rbcS2 intron 1 is known to contain an enhancer sequence and can significantly improve transformation efficiency. All of these components were engineered for greater transformation efficiency, which was evident in the number of transformants every transformation yielded (Berthold, Schmitt, & Mages, 2002).

C. Mutants Generated by Insertional Mutagenesis

There were 35 mutants whose sequences had been aligned using the BLAST services of NCBI and JGI Chlamydomonas Genome Portal. Of all the mutants, only 12 had sequences that aligned with chromosomal/gene sequences. These mutants had fragment

insertions into genes that were responsible for essential enzymes. While disruption of flagellar genes such as ODA1 and ODA14 may be more directly linked to a motility defect, it is entirely possible that even the disruption of an essential enzyme can affect flagellar motility. One possible scenario is that of the DR2BA4 mutant. It has a palmelloid phenotype, and BLAST alignment shows that the fragment integrated near chromosomes 3 and 15, both of which contain genes responsible for the production of serine-threonine/tyrosine protein kinase. Protein kinases are responsible for phosphorylation of proteins, and are known to regulate cellular pathways. It is possible that the reason why this mutant has a palmelloid phenotype is due to a dysfunctional protein kinase, which may have been necessary to phosphorylate a protein that ultimately would signal to outer dynein arms to have the microtubules slide against one another, which could have resulted in flagellar movement. This is currently a theoretical scenario but one that could be a possible explanation for this mutant. Further research will need to be done to understand how disrupting genes responsible for enzymes contributes to a flagellar motility defect.

D. DR10-3c 4A9: Disruption of ODA1

The disruption of the ODA1 gene resulted in diminished amounts of DC2 and DC3 proteins, both docking complex proteins necessary for the outer arm dyneins to bind to a microtubule. Since DC3 is made in the ODA14 gene locus, disruption in the 5th exon must have been sufficient to affect protein production. It is very possible that disruption of the DC2 protein production can also affect DC1 and DC3 protein production, as all three are necessary for outer dynein arm binding to a microtubule. One possible scenario for this is that it interrupted the enhancer sequence that controls the ODA3, ODA1 and ODA14 gene loci, all of which make docking complex proteins. Disrupting the enhancer that controls the activity of all three genes could result in all three having truncated or diminished proteins even though the marker only immediately affected ODA1. To this end, the Western blots

should also have been probed with DC1 protein to see if it was affected as well. Further experiments would have to be done to determine why disruption at that site resulted in diminished amounts of DC2 and DC3 protein. Another follow-up for this mutant would be to determine the downstream flanking sequence where the marker had inserted. This would give more insight into why insertion at that site caused a flagellar motility defect as well as the reason for a diminished amount of DC3 protein.

E. Swimming Velocities of the Ca^{2+} ATPase Mutant

The swimming velocities collected on the BG8 mutant showed that both calcium as well as the absence of it had negative effects on the ATPase mutant. It is interesting that overall swimming velocities did not improve with the absence of calcium. Since the function of Ca^{2+} ATPase is normally to regulate the amount of calcium inside the cell, it can be hypothesized that the Ca^{2+} ATPase is unable to regulate the amount of calcium in the cell, but this theory only applies to the cells exposed to calcium. For this mutant, it is possible that the calcium ATPase is unable to handle exposure to calcium and regulate calcium levels within the cell. As for the BG8 cells exposed to no calcium, could it be that cells are unable to function this time due to the complete absence of calcium? This is a possibility, as BG8 cells grown in the nutritional growth media (contains a small amount of calcium) seem to have overall faster swimming velocities. Overall, further research would have to be conducted to understand why calcium ATPase is unable to regulate the amount of calcium in the cell, as well as how dysfunctional calcium ATPase results in a twitchy phenotype.

V. Conclusions

Insertional mutagenesis of *Chlamydomonas reinhardtii* has resulted in a collection of mutants with various phenotypes and insertion sites. The next steps for this project would be to continue genetic analysis on the ODA1 and Ca^{2+} ATPase mutants as well as refine the methods for insertional mutagenesis so as to improve efficiency and mutant throughput in addition to generating more flagellar motility mutants. A library of *C. reinhardtii* flagellar mutants with identified insertion sites and flagellar motility defects is the ultimate goal of this project, and these were its beginnings.

VI. Bibliography

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VII. Appendices

A. Mutants Generated by Insertional Mutagenesis

Mutant Strain	Hyg-specific primer	Deg. primer	Phenotype	RESDA sequence	NCBI BLAST	JGI BLAST	BLASTp
1a	UP	Sac	Shaky, jerky swimming	Sequence File : DR1aUPSac-UPS.seq >DR1aUPSac-UPS_E06.ab1 NNNNNNNNNNNNNNNNNNNNAGCGG ANTGNGATCACAAGCTCGAGTGGCCTGTG TAGAAGTGGTAGTGATCTAGGTGTT TGAATATGGCTTTGGTAGCTCGCTATAAT GTCTTTGCAATCGGGGGCCTGGCTATTTA AACAGCGCTCGCCCTGGAGCGG CATCGGAGCGCCCATGCAGCCCCGAAGGA GCTTCGGGGGGTCGAAGCATCATCGGTGT TGCATGCAGCGCCGGGAAGCCG TCTCGCAGCCCGCCCTACCTTTTGCTGGA AGTGTCATAGCGCAAGAAAGAATGCGGCT GTGCCCCAGCTCCGGCGGTGGC AGCAGCCTAACGTCCTTCACCGCGGCCCC CCCCCGTCACTCTGCTCACTGGAACNTGC NNNCACTGACTGCAACAAATGT AGATGATTGANACGGGAGGCANACCNNN CTGACTTCAACACCAATTCTATTTGCNTT GNCNTATCATNTTCAGACTAGTC TNTGCTTTCTTATTATNACATGTGCGTGT CTGTGNNCCAACCCGTAANTCATACGCAG AGTGAGNTGNTCNNNNNCCTCA CCTNNNTCANNGAACNGANNNNANACA TCANATGACTTANGNTATACTTTNANNT TAAATCNTTCCCTTCAANTGNCAGA NNNAANNNNNCNNGCNGNTGCNNCCGN GCTGCAGATGACANATCTCGACTTATCTN TATGNATNGAGCATTGNAANAAAA GNATAGCATTGCTAATCTATTTGTCGCTN CNNNNNNNTATCTATCANTNTAAATCA NTATTTNTNTCCANNCGCAGNNNN NCTCGTGNCTCANGATCTGTGTCNGNNC TTTGNANCNGATANANNATATCNNCCTG NAGACCAATATGTCTGCCTGCNTA NATNANNANACAAGCNNGNTTATNANN NATNTTCATCGNNNANNATCGNCNNGN CNNTNANNTNNNAANNATGATGCNGC NNNTANANNCCGNACATGNTNGNCNNG NCGAANNNTGNNNNANNANNGANGCNG CANNNAANNNNNNGNNNNNNNANNNC GCNNGNAGCCNNANNNGTNGTNNNAGN ANNNNAAAANGGCCGNNNNCCANTNAT NGCTTNNNNANNNGG	B-tubulin promoter	IN GENE Chr. 9 C-type lectin Peptidase, trypsin-like serine & cysteine	PKD2 C. reinhardtii flagellar protein
1a	DP	Taq	Shaky, jerky swimming	>DR1aDPTaq NNNNNNNNNNNNGNNNNNGNNNNGAN GACGGCGGTGGANGGAAGANACTGCTCT CAAGTGCTGAAGCGGTAGCTTAGCTCCCC GTTTCGTGCTGATCAGTCTTTTTCAACAC GTAAAAAGCGGAGGAGTTTTGCAATTTT GTTGGTTGTAACGATCCTCCGTTGATTTT GGCCTCTTTCTCCATGGGCGGGCTGGGCG TATTTGAAGCGGGTACCGGGCCCGTCATC CCATGGAAGCTGCTGACGGCATTGGGCGG AGAGCCAGACACCGCCACCGTGCTCCCCT GCAGCAACACGAGCAGCGACAACAGGAGC ACAAGGCCACACGAGCTCTCAGGCATGCG ACACCACTTTTCGTGCCTGTTCGACTCCC CCCCCCGTCACTCTGCTCACTGGAAAGAT GTTTGAGACANCTAGCACATATGTCTGTG TTCAACATCTATACTATTTGNNTTATCTC AGACATATGAGTTTTGGCCCAAGCNTTTC CGGAGGAACTGTGCGGTCCTTGTGGGGGA CGGCGGCCCCCCCCCCTCATAGNAACATG CCCNNCTCAACAACATANNCCATAAAAC TGANGACNNCCCCANTANATGGNGTAAN NNNNNANTTATTTTTNNTANNTACTNN CNATTGNTTNTCCTGACTCAAGNNGNNG TTCNTTTNNCAACGNACNCNAGATAANT ANACCCACNTTTCTTGTANNNNNNNNN	Rubisco 3' UTR	Half exon, half intron Chr. 9 C-type lectin Peptidase, trypsin-like serine & cysten	

				NNATGACANNANAGCAATGCNTAGCNAT TTNGNNGCGCCGGANGGNANNCGATCAG TNCNNANTNNACTCNTNGATTNTNNAG TTCGCNATNC			
2a	UP	Pst	Most cells don't move, some barely but shaky	> DR2aUPPst NNNNNNNNNNNNNCNNNTNAGCGGA GTGNGATCACAGCTCGAGTGGCCTGTGTA GAAGTGGTAGTGATCTAGGTGTTTGAAT ATGGCTTTGGTAGCTCGCTACGATCGCCA CGAACATTGCATGCGTCCCCACCCCGCTC CACAAGAGCTCTCGGCCACTATTGCTGCA GCAGCTGCACCCTGGTGTTTGTACACACA GGACAGCCCATCGTGTGCTGTTGCCACAA GCAATAGAATCAGCACCATCCCCTTGCAC AATCACATACCGNACTGCTGAATGAGGGT TGATCGGGTGATCAGCCTCCCCGCTTGTT CAGGCACCGCCCCCCCCCCCCCCCCCCCC CNGGGTNGGGANGGCNNNCCCCCNTTGG NAAAAAAAANTTGGGGGGGGGGGGGGG GGNAAAANNNCTTTNGGNNNCNAAANN NNNCCCCCNNNNTNNNAANCCNGGNCCC CGGCCCCCCGGNNCCNNAAAAAANNTAA ANNNANTTTTNNNGNNNNNAAAGGGGN NNTTNNGGGCCCGGACCCNNNNGNNGGG GGGGNNANNNNGGGGGNNNGGGGGNTT TNGNNAGGGTTAACNCCCCNANNNGGGN NNNCNNNNNNGGGGNNNCCNNCCCCCC AAAATCCCNTNNNAAAACCNNNTNGGN NNCTGNNNNAAGNCCCCCANNCCCCANN NNNNANAAAANNNNANNGGNNAANNN NNNNANCNNCCTNCNNNNNNNTTNNNT TNNCCNNNNNNNNNNNNNANNTTNGG GGNNCCNNTTTNNNNNTNNCNNCCNNN NNNNCCCNNNNTNNNCAANNTNNNNN NNNNNNTTTNTTTTTNNNNNNNNNNN TTNNNNNTTTNNNNNGNNNNNCCCCCN GTNNNNNNNNTNNNNNNNNNNNNNNN NNNNNCNNNNNCNNTNNTNNAANNNN ANNNTNGNGNANCGNNNANNNNNNNA TNNNNNNNNANNNNNNNNNNNNNN NNNNNNANNNNCTNNNNNNNNNNCNN CANNNNNNNNTNNNNNNNCNNNNNAA NNNNGNTCNNNNNNTNNNCTNANANT NNNNNNGNNCNTNNCATGNNGACNNNT NGANTGNCNNNNNNNNNNCNNNNNAN NNNNTNNNNCNNNNN	B-tubulin promoter	Chr. 12 Dysferlin	
2a	DP	Alu	Most cells don't move, some barely but shaky	>DR2aDPAlu NNNNNNNNNNNNNNNNNNCNNNNNAN AGNGNNTNNNNGAGANNGNGGGTCCCC TGGGCNGGAGCGGAACCTAACCCCCCCTN TTCNNGGAANGAATCTTTTTCCTTACNN AAAAGGGGAGGGTTTTTGGNGNTTTGGT GGTTGGGCGANCCTCCGTCNATTTTTGCC GCTTTCTCCTGGGGGGGCTGGGCTGTTTT AAGAGAGCACCGGACCCGACATCCTCATG AACCTAGGNGCCCGGCCNCTTGGCATTNG CCTGCCCCNNNGCAAAGGTNANTGGCTA CGGGNCGTGTGNCACAGACGGGCAACAGC NAAGCCAATTGCNNACTGCACNGCACCN CNAAATGNGNNTGAAATCANNNGCCGCG TGGNNTGTCTGTCACTGCCATGACAATTG GGTTGGCACAGACATAACGCTTGAATCAC CACCCANCCCATGGTCCTCGGCNNACCTN NGACACTGGAATNTCGGCATTTCCTATTA TNTGATTGCGGNCGTANCTGCATGGNNA AAACAACGGNTTGCTNAGTGGAGCTTGC GCTCACTTACTCTATAATCCTAGCGACNA ACTACAAAGTGTGTGTGNTGCAACNAAT AGATGAGCAGCTGCTGCANCCTTTCANNT GNCNNNANNCANNGNNNNNNANAGTAG CTGANNTCACAACTCGACANATCTATAT NNNNTTNTNNNNNNNNNNGGNAGCCCT ATCGNNTNNACATTGCNTCTCNNTCTGN TANCCNGCTGTCGTAACTGNNCNGNTNN TCCTGNNATCNCTGCNTGCATNNTCNNN NNAGNTCTGGNTNNNNTNNTCNGNNAC CCAATNANNTNAGANCTGCTTATCANAN AGTANNNCANCGNGNGCTATGANGNCGT	No significant similarity	No hits	

				ATNCCATNCNNGACATNGTNNCAGNCNG NGATGTAATCNNCCATATNTNNGATGGA NACNTNGAGGNCTGCAATTNNNNNCNN NNNNNNCCNNANNANCNNGNNGNNTA ANATGTGCNTNGNTNGAANNGANNACC CNNNCANNNNCCANNNNNNNTTNCNNN NATGNGNNGNNNGNNNGCNNCTNANNA NNTATCNTNANNNANNNAAATGNNACG ACTACANNGNNNNACNNNATNNNNNGC NNNNNCNNNNCNNCNNNNNNNNNNTT NNNNNNTNNNNTNNGNACGNNNNNNN			
3g	UP	Taq	Stop & start swimming, spinning	>DR3gUPTaq NNNNNNNNNNNNNNNNNTTAGCGGAN TGNGATCACAAGCTCGAGTGGCCTGTGTA GAAGTGGTAGTGATCTAGGTGTTTGAAT ATGGCTTTGGTAGCTCGCTATAATGTCTT TGCAATCGGGGGCCTGGCTATTTAAACAG CGCTCGCCCTGGAGCGGCATCGGAGCGCC CATGCAGCCCCGAAGGAGCTTCGGGGGGT CGAAGCATCATCGGTGTTGCATGCAGCGC CGGGAAGCCGTCTCGCAGCCCGCCCTACC TTTTGCTGGAAGTGTCATAGCGCAAGAAA GAAGCTTGATATCGAATTCGTAATCATGG TCATAGCTGTTTCCTGTGTGAAATTGTTA TCCGCTCACAATTCCACACAACATACGAG CCGGAAGCATAAAGTGTAAAGCCTGGGGT GCCTAATGAGTGAGCTAACTCACATTAAT TGCGTTGCGCTCACTGCCCGCTTTCCAGT CGGGAAACCTGTCGTGCCAGCTGCATTAA TGAATCGGCCAACGCGCGGGGAGAGGCGG TTTGCGTATTGGGCGCTCTTCCGCTTCCT CGCTCACTGACTCGCTGCGCTCGGTCGTT CGACTCNCCCCCCCGTCACTCTGCCTCACT GGA	S. cerevisiae aquaporin gene	Chr. 12 B-tubulin	
3g	DP	Taq	Stop & start swimming, spinning	>DR3gDPTaq NNNNNNNNNTGGCNTGCNNNTGNCGAC GNNGANGGATGGAAGATACTGCTCTCAA GTGCGGAAGCGGTAGCGTAGCTCCCCGTT TCCTGCTGATCAGTCTTTTTTAACACGTA AAAAGCGGACGAGTTTTAGAATTTTGTT GNTTGGACGATCCTCCGTTGATTTTGNCC TCTTTCTCCATGCCCGTGCTGAAAGAATT TGAAGCGNCGACCGGGCCCGTCATCCCAT GAAATCTTCTTTCTTGCCCGATTTTACTT CCAGCAAAANGAAGGNNGGCACGAAACA CGGAGTCCCGCCACTGCATGNGACCTTGA GAGCGGTTCCATCCCTCGAAGGCCCTTCG GGAGGGCATGAGCGCCCCAATGCCGAGCG CCCCCTTTTATTCNNANNAGGGAGGAGT CAATGGACTCCTCTTGACGAGCACTATCA CGCTGTACCATCGGCGGTCCTGTTCTGAG ACTTCGGTCGGTGCGTCAGCAGGTCCCTG CAATGCAATCTTCGGTCACATGCGGGATC CTATCCATGGAAATCCCTTCGACACNNCC CCCCGTCACTCTGCTCACTN	Only hit: CC-503 strain	Chr. 12 Protein kinase Ser-thr protein kinase Chr. 16 Chr. 1 Endoplasmic reticulum protein ERp29 Protein kinase Ser-thr protein kinase Chr. 10 Chr. 6 Histone H2A Chr. 17, 9, 14, 13, 2	
8a	UP	Taq	Slow swimming, twitchy	>DR8aUPTaq NNNNNNNNNNNNNNNNNNNNNNAGCG GANTGCGATCACAAGCTCGAGTGGCCTGT GTAGAAGTGGTAGTGATCTAGGTGTTTG AATATGGCTTTGGTAGCTCGCTATAATGT CTTTGCAATCGGGGGCCTGGCTATTTAAA CAGCGCTCGCCCTGGAGCGGCATCGGAGC GCCCATGCAGCCCCGAAGGAGCTTCGGGG GGTCGAAGCATCATCGGTGTTGCATGCAG CGCCGGGAAGCCGTCTCGCAGCCCGCCCT ACCTTTTGCTGGAAGTGTCATAGCGCAAG AAAGAAGCTTGATATCGAATTCGTAATCA TGGTCATAGCTGTTTCCTGTGTGAAATTG TTATCCGCTCACAATTCCACACAACATAC GAGCCGGAAGCATAAAGTGTAAAGCCTGG GGTGCCTAATGAGTGAGCTAACTCACATT AATTGCGTTGCGCTCACTGCCCGCTTTCC AGTCGGGAAACCTGTCGTGCCAGCTGCAT TAATGAATCGGCCAACGCGCGGGGAGAGG CGGTTTGCGTATTGGGCGCTCTTCCGCTT	S. cerevisiae aquaporin	B-tubulin	

				CCTCGCTCACTGACTCGCTGCGCTCGGTC GTTCGACNCNCCCCCCCGTCACTCTGCTC ACTGGA			
AF10	DP	Sac	Most cells not moving Cells that do move very slowly/ Can't swim straight	>drAF10DPsac5-DPS_A11.ab1 NNNNNNNNNNNNNNNNNNNNNGAC GACGGCGGTGGATGGAAGATACTGCTCTC AAGTGCTGAAGCGGTAGCTTAGCTC CCCGTTTCGTGCTGATCAGTCTTTTTCAA CACGTAAAAAGCGGAGGAGTTTTGCAATT TTGTTGGTTGTAACGATCCTCC GTTGATTTTGGCCTCTTTCTCCATGGGCG GGCTGGGCGTATTTGAAGCGGGTACCGGG CCCGTCATCCCATGGAAGCTTT CGTCACGACATTGAGTTGCCTGCGTTCTC CGGGGACTGTGAGGCAGAGATGTGCTTGC TGTGCAAGCCTGGAAAGCTCTG AGAGCTACACGGACATGGCTGGCGCGGAG CGCACGCCCTTGGGCCGTGGTGGCGATAG CATCCGAAAAATGTGGTCGCAG CCAGGGGTTTGGGCGCGACATCCCCTTCC TTGCCGAAACTTCCCCTACAACGAGGGGG CTAAATTGCTACAAATTTACAG GGGCTGCATGGAAAGAACACACGGACACG CGGCCTTGCCCATACTCCGGCCTCGGCAC AAGAGCTGCGTGTGCATCGTGA AAGTACATTGCATCGTCTACGTCGTGAGT CCTGATACCTGTTCGAGTTTCTTCCTTTC TTCCTTCCGCGCTGTGTTTCTT GGCTGTCTTGTTACTCGCGAGTGAATGTT TGTGACTTTGCAGCGCAATCTTAATAGAA CTTGTAATTCTACGCCCTCAAC ATGAGCATTCTACTTGAATAGGCATACCG TTTAAAATTGCAACATGCCTCAGCAGTTC CGCTTGAAACCGCGGCCCCCCC CCGTCACTCNGGNCTNNCACTGGN		Rubisco 3' UTR Chromosome 5	Kelch repeat-containing protein / kelch motif Tip elongation aberrant protein Dynein heavy chain [E. siliculosus] 32, 27% identity
DR2 AH11	DP	Sac	All cells in clumps	>dDPsac8-DPS_B11.ab1 NNNNNNNNNNNNNCNNNNNNNGACG ACGGCGGTGGATGGAAGATACTGCTCTCA AGTGCTGAAGCGGTAGCTTAGCTCC CCGTTTCGTGCTGATCAGTCTTTTTCAAC ACGTAAAAAGCGGAGGAGTTTTGCAATT TTGTTGGTTGTAACGATCCTCCG TTGATTTTGGCCTCTTTCTCCATGGGCGG GCTGGGCGTATTTGAAGCGGGTACCGGGC CCGTCATCCCATGGAAGCTTCT TTCTTGCGCTATGACACTTCCAGCAAAAG GTAGGGCGGGCTGCGAGACGGCTTCCCGG CGCTGCATGCAACACCGATGAT GCTTCGACCCCCCGAAGCTCCTTCGGGGC TGCATGGGCGCTCCGATGCCGCTCCAGGG CGAGCGCTGTTTAAATAGCCAG GCCCCCGATTGCAAAGACATTCGCCCGCA GCCGGCTCGCGCCTACGCCCGAGCTTCTA GCCGATGTTCGCAAGTGCTGGC GCTGCCGCTGCCGCTGCTCTTGCCGGTGT TGCTGGCCCTGTAGATGCTGCTGGTGCTG GTGCTGCTGCAGCTGCCGCCGG TCCTGACAGCCGGTACCTTGTGGCTCCGC GGCCCCCCCCCGTCACTCTGCTCACTGG	Rubisco 3' UTR	Chr. 2 rbcS2	
DR2 AB11	DP	Sac		>drAB11DPSac10-DPS_C11.ab1 NNNNNNNNCCNNNNNNNNNNNNNNN NNNNGNNNNGCGGTGGATGGAAGATAC TGCTCTCAAGTGCTGAAGCGGTAGCTT AGCTCCCCGTTTCGTGCTGATCAGTCTTT TTCAACACGTAAAAAGCGGAGGAGTTTTG CAATTTTGTTGGTTGTAACGAT CCTCCGTTGATTTTGGCCTCTTTCTCCAT GGGCGGGCTGGGCGTATTTGAAGCGGGTA CCGGGCCCGTCATCCCATGGAA GCTTCTTTCTTGCGCTATGACACTTCCAG CAAAAGGTAGGGCGGGCTGCGAGACGGCT TCCCGGCGCTGCATGCAACACC GATGATGCTTCGACCCCCGAAGCTCCTTC GGGGCTGCATGGGCGCTCCGATGCCGCTC CAGGGCGAGCGCTGTTCCGCGG TAGGAGAGTCGCGACGAGGAGACCGAAAC CAGCACCACGCAGGGCGCACCCGTAGCTA	B-tubulin regulatory region Rubisco 3' UTR	Chr. 2 rbcS2 Chr. 12	Kinesin in leishmania

				GTGCCTCAGAGACGGCTCTGAC GCCGCGCAGCAAGTCAGTGACANCGGCGC TGTCTCCGCTGGCTTCGGGACCGGTGCCC CCGACAGAAAGCCCGGCGGCGG AGGCAAGTTCGGGACTGGGGCGCACAGGC AACAGCAGCAGCAGTACGACCGCGGCCCC CCCCCGTCACTCTGCTCACTGGA			
DR2 BA4	DP	Alu	All cells in clumps	>drBA4DPAlu9-DPS_D11.ab1 NNNNNNNNNNNNNCNNNNNNNGACGA CGGCGGTGGATGGAAGATACTGCTCTCAA GTGCTGAAGCGGTAGCTTAGCTCCCCGTT TCGTGCTGATCAGTCTTTTTCAACACGTA AAAAGCGGAGGAGTTTTGCAATTTTGTTG GTTGTAACGATCCTCCGTTGATTTTGGCC TCTTTCTCCATGGGCGGGCTGGGCGTATT TGAAGCGGGTACCGGGCCCGTCATCCCAT GGAAGCTTGGC ACTGGCCGTCGTTTTACAACGTCGTGACT GGGAAAACCCTGGCGTTACCCAACTTAAT CGCCTTGCAGCACATCCCCCTT TCGCCAGCTGGCGTAATAGCGAAGAGGCC CGCACCGATCGCCCTTCCCAACAGTTGCG CAGCCTGAATGGCGAATGGCGC CTGATGCGGTATTTTCTCCTTACGCATCT GTGCGGTATTTCACACCGCATATGGTGCA CTCTCAGTACAATCTGCTCTGA TGCCGCATAGTTAAGCCAGCCCCGACACC CGCCAACACCCGCTGACGCGCCCTGACGG GCTTGTCTGCTCCCGGCATCCG CTTACAGACAAGCTGACNNCCCCCCGTCN NNNNNCNTCACTGGA		Chr. 2 Rubisco 3' UTR	
DR2 BH7	DP	Pst	Swimming cells very slow, some shaky	>drBH7DPPst7-DPS_E11.ab1 NNNNNNNNNNNNNNNNNNNNNNNNG ANGACGGCGGTGGATGGAAGATACTGCT CTCAAGTGCTGAAGCGGTAGCTTAGCT CCCCGTTTCGTGCTGATCAGTCTTTTTCA ACACGTAAAAAGCGGAGGAGTTTTGCAA TTTTGTTGGTTGTAACGATCCTC CGTTGATTTTGGCCTCTTTCTCCATGGGC GGGCTGGGCGTATTTGAAGCGGGTACCGG GCCCGTCATGGCCCATCACTCC CCGCGCCCCGGCCCCACCCCAACAAACCCC AACATGGCCCCACCCCAACAAACCCCAAT ACGGCCCCACCCCAACAAACC CCGGCGGTACGGCCCCACCTCTACGGCGT CTCCCACGGCGAAGATGGCTGGGTCACTG GTGCGCATGTGGGCGTCCGTCT TGATGCCGCCGCGCCCGCCCAGCTCCAGA CCCGCGGCCTTGGCCAGGCTGGTCTCCGG CTTGACGCCGATGGCCTGCATT CGGGCGCAATCACACACGCAGCAGCAGGT TTCCGTCCTCCCCTCTCACCCCACCCACGT GCATCGGCAAGCAGCTTGACG GCACGTTTGGGGCCATTTGGCAATCCGGC TCCTCTCTTCACCCCTTGCCACCCCTCTGC AGNNNCCCCCCGTCACTCTGC CTCACNN	Rubisco 3' UTR	Chr. 2 rbcS2 chr. 6 FAD/NAD-linked reductase Pyridine nucleotide-disulphide oxidoreductase	NADH oxidase FAD-dependent Pyridine nucleotide-disulphide oxidoreductase - highly conserved in bacteria CoA disulfide reductase
DR2 BA4	UP	Sac		>drAB4UPSac12-UPS_F11.ab1 NNNNNNNNNCNCCNTAGCGGANGCGGN NNNNAGGGGTAGAAATGGAAGCGGCAAA ATCATAGTATGGTCAAAAGGTCAGC GGATAGTTAGAGATTGCATCGGTGCCACG AGGGACCTGCTGACGCAGCTCACGCGCCA GCGAACACGCACTGCGGTAGTA GTACGGACGCCGTGCTAGACCTCGCTCAG GGGCGTTAGAGCGCAGGTTACAGCGGATG ATTGACAGCTGGGAGAGGATGA AGTTTTCCAGATCAACGTCAGGTGAGCCT CCGTCTGGCATAAACCACCACCCCGGGGG TTCCGGTGATATGGCATTAGGC AGGCAATGCCCGCCCATCCACCCGCGGCA GCCAGTCGTGTAAAGGCCCCGTCACGCGG AAGGTCGTGACTCGGGAACCTC AGCACCTCAGAGCTCAGGTGCATAGTTTC CACAACACTAAGCATCCTGGCGTGCAGCC CAGGTGCGCAGGGAAGGCGTCA CGAAACTCTGCCCCTCACTCAGCATGAAT GCGGCACCGCGGCCCCCCCCCGTCACNTN	No significant similarity	mid-chr. 14 (intron) mid-chr. 1 (exon) mid-chr. 6 (exon) mid-chr. 6 (intron) mid-chr. 3 (exon) Ser/Thr, Tyr prot kinase Chr. 3 overlap (exon) Glycogen/starch synthase Glycosyl transferase	Zinc finger prot (equus) Ca^{2+}-binding caleosin Prot kinase, ser/thr Starch synthase Glycosyl transferase Mixed-lineage prot kinase 1 (Homo sapiens) Mitogen-act prot kinase (mus musculus)

				NTCACTGGANANNNANNCTCTG CGCTCTNTGCNNNGNNGCCNGAGCGGTA TCATCTGACTCAAAGGCGGTAATACNGNT ATCCACAAAAATCNNGGGATAAC GCANGAAANAACATGTGANCNNANNNG NCNNCANNNGCCATGGAANCGTAAAAAG GNCGGGTTAGCTGNCNTTTTTTCCA TANGCNCCNCCCCCCCTGACGATNNNNN NNN		mid-chr. 15 (intron) ser/thr, tyr prot kinase chr. 10 (not in gene)	
DR2 BA4	UP	Alu	All cells in clumps	>drBA4UPAlu9-UPS_G11.ab1 NNNNNNNNNNCNCCCTTAGCGGAGTGC GATCACAAGCTCGAGTGGCCTGTGTAGAA GTGGTAGTGATCTAGGTGTTTGAATATG GCTTTGGTAGCTCGCTATAATGTCTTTGC AATCGGGGGCCTGGCTATTTAAACAGCGC TCGCCCTGGAGCGGCATCGGAGCGCCCAT GCAGCCCCGAAGGAGCTTCGGGGGGTCGA AGCATCATCGGTGTTGCATGCAGCGCCGG GAAGCCGTCTCGCAGCCCGCCCTACCTTT TGCTGGAAGTGTCATAGCGCAAGAAAGA AGCTTGATATCGAATTCGTAATCATGGTC ATAGCTGTTTCCTGTGTGAAATTGTTATC CGCTCACAATTCCACACAACATACGAGCC GGAAGCATAAAGTGTAAAGCCTGGGGT GCCTAATGAGTGAGCTAACTCACATTAAT TGCGTTGCGCTCACTGCCCGCTTTCCAGT CGGGAAACCTGTCGTGCCAGCTGCATTAA TGAATCGGCCAACGCGCGGGGAGAGGCGG TTTGCGTATTGGGCGCTCTTCCGCTTCCT CGCTCACTGACTCGCTGCGCTCGGTCGTT CGGCTGCGGCGAGCGGTATCAGCTCACTC AAAGGCGGTAATACGGTTATCCACAGAAT CAGGGGATAACGCAGGAAAGAACATGTG AGCAAAAGGCCAGCAAAAGGCCAGGAACC GTAAAAAGGCCGCGTTGCTGGCGTTTTTC CATAGGCTCCGCCCCCCTGACGAGCATCA CAAAAATCGACGCTCAAGTCAGAGGTGGC GAAACCCGACAGGACTATAAAGATACCAG GCGTTTCCCCCTGGAAGCTCCCTCGTGCG CTCTCCTGTTCCGACCCTGCCGCTTACCGG ATACCTGTCCGCCTTTCTCCCTTCGGGAA GCGTGGCGCTTTCTCATAGCTCACGCTGT AGGTATCTCAGTTCGGTGTNNTCGTTCGC TNNNCTGANNNCCCNNNNNNNNNNNN NNNNNNNNNNNNNNNNNCCNNNNNNN NNNNNNNNNNNNNNNAANNANNNNNNN AANNNNNNNNNNNNNTNGCNNNTNNNN NNNTNNNNNGNNNNNNNN	Aquaporin (Aqy1) gene, S. cerevisiae	B-tubulin	B-tubulin
DR2 BH7	UP	Taq	Twitchy, shaky swimming	>drBH7UPTaq7-UPS_H11.ab1 NNNNNNNNNNNNNNNNNAGCGGANTG CGATCACAAGCTCGAGTGGCCTGTGTAGA AGTGGTAGTGATCTAGGTGTTTGAA TATGGCTTTGGTAGCTCGCTATAATGTCT TTGCAATCGGGGGCCTGGCTATTTAAACA GCGCTCGCCCTGGAGCGGCATC GGAGCGCCCATGCAGCCCCGAAGGAGCTT CGGGGGGTCGAAGCATCATCGGTGTTGCA TGCAGCGCCGGGAAGCCGTCTC GCAGCCCGCCCTACCTTTTGCTGGAAGTG TCATAGCGCAAGAAAGAAGCTTGATATCG AATTCGTAATCATGGTCATAGC TGTTTCCTGTGTGAAATTGTTATCCGCTC ACAATTCCACACAACATACGAGCCGGAAG CATAAAGTGTAAAGCCTGGGGT GCCTAATGAGTGAGCTAACTCACATTAAT TGCGTTGCGCTCACTGCCCGCTTTCCAGT CGGGAAACCTGTCGTGCCAGCT GCATTAATGAATCGGCCAACGCGCGGGGA GAGGCGGTTTGCGTATTGGGCGCTCTTCC GCTTCCTCGCTCACTGACTCGC TGCGCTCGGTCGTTCGACTCCCCCCCCCGT CACNTGNCTTCACTGGANNAANANATNN TTNCNCNATNNNAAACNNNTCN NGAANGAATGTGAGANTAAANCCCNTCC AAANCGNAATGGACNGNACCCAGGCAGN AAANNTAACGNATTANCTACNGCN CCNTCNNCNNGAAAANACTAGANAAATA TANCGAAAAGATGATNGGGCTAACCCAT	Aquaporin (Aqy1) gene for S. cerevisiae	B-tubulin	B-tubulin

				CNGAAAGACGTGTCGATACCCCNT TNAACTCNAGACGNACTGACCCATTGNC AAATGGTGAACCAAGTCCAAAGATNNAA NNGGCTATCTGNNCNNNATTTTAN GNTCGNANAAATNAATCTANTCACAGAA TC			
DR2 BC11	UP	Taq		>drBC11UPTaq4-UPS_A12.ab1 NNNNNNNNNNCNNNNNTAGCGGANTGC GATCACAAGCTCGAGTGGCCTGTGTAGAA GTGGTAGTGATCTAGGTGTTTGAA TATGGCTTTGGTAGCTCGCTATAATGTCT TTGCAATCGGGGGCCTGGCTATTTAAACA GCGCTCGCCCTGGAGCGGCATC GGAGCGCCCATGCAGCCCCGAAGGAGCTT CGGGGGGTCGAAGCATCATCGGTGTTGCA TGCAGCGCCGGGAAGCCGTCTC GCAGCCCGCCCTACCTTTTGCTGGAAGTG TCATAGCGCAAGAAAGAAGCTTGATATCG AATTCGTAATCATGGTCATAGC TGTTTCCTGTGTGAAATTGTTATCCGCTC ACAATTCCACACAACATACGAGCCGGAAG CATAAAGTGTAAAGCCTGGGGT GCCTAATGAGTGAGCTAACTCACATTAAT TGCGTTGCGCTCACTGCCCGCTTTCCAGT CGGGAAACCTGTCGTGCCAGCT GCATTAATGAATCGGCCAACGCGCGGGGA GAGGCGGTTTGCGTATTGGGCGCTCTTCC GCTTCCTCGCTCACTGACTCGC TGCGCTCGGTCGTTCGACTCNNCCCCCCG TNNNNNNNNNNNCCACTGGANNNNNNC CCGNGTGTTANTTCNNNNNNNNNN NANNNTNNCNNGNGNNNNGNNNGNNG ANTTGNNCGNNGNTNNNNTANANNNNA NN	Aquaporin (Aqy1) gene from S. cerevisiae	B-tubulin	B-tubulin
DR10-3a BD4	UP	Sac	Swims at normal speed with slight shakiness Lots of oblong-shaped cells	>dr103abd4UPSac NNNNNNNNNNNNNNNNNNNNNNNN GNANTGNGATGACNNNTCGANNNNNNN NANNNNNNNANNGNNNNGNNNNNNNN NN NNNNNNNNNNNNNANNNTAGAAACAA NGANCCNNTNAANAAAGNCGTANNAAT GAAGGAAGGCAACANTGCCCCAANCNN NGACAACGNNGATGGGCAANNCNANNN AAANNAGNANNNNAAGTNAAATGNGNA GAAGCNACTACCNNNNAAAAAAAAAG AAAAAAAAAAAAAAAAAAAAATTNNTGG ATNGTCNGCNATCTCCCGGGAACACGCCN TGCAAACAAATGNNNCCCATTTN NTTTCANTNNCTGTTGACCCNNNGNGGG ANGAAN	No significant similarity	No hits	
DR10-3a BD7	UP	Sac	Swim at normal speed but not straight, slight shakiness	>dr103abd7UPSac NNNNNNNNNNNNNNNNNNNNNNNN NNNNNNNNNNNNNNNNNNNNNNNN NNNNNNNNANNNNNNNNNNNNNGN NNNNNNNNNNNNNNNCNNNNNNNNA NNNANANCNNNNNNNNTNNTTAAGAGC CNNACNNTNTGCTGAANNCCNCNACANC ATNNNNNNCNNNNNNCCNNNNNNTTA TTTNN	No significant similarity	No hits	
DR10-3a BD11	UP	Taq	Swim slightly slower, shaky	>dr103abd11UPTaq NNNNNNNNNNNNNNNNNNNNNNNN NNNNNNNNNNNNCAANNNNGANNNNN NNNNGNNNNNNNNNNTTTNNANNTNN NNNNNNNNNNNNNNNNNNNNNNNCNC GCCCNNNGAAGGCGCNNNNNCTNNNAG CNNNGCCAAAATGCCCCAANCCTGATAA CAATNCCGNNTNNANAACCTNGNTTNN NNGNCNGAN	No significant similarity	No hits	
DR10-3a BE3	DP	Pst	Swim slowly	>dr103abe3DPPst NNNNNNNNNNNNNNNTNNNNNNGACG AACGGCGGTGGATGGAAGATACTGCTCTC AAGTGCTGAAGCGGTAGCTTAGCTCCCCG TTTCGTGCTGATCAGTCTTTTTCAACACG TAAAAAGCGGAGGAGTTTTGCAATTTTG TTGGTTGTAACGATCCTCCGTTGATTTTG GCCTCTTTCTCCATGGGCGGGCTGGGCGT ATTTGAAGCGGGTACCGGGCCCGTCATCC CATGGAAGACCGGTTGGCAGCCCGCAGCC	Rubisco 3' UTR	Rubisco 3' UTR Chromosome 3 intron	Paf1 complex component Rtf1, homolog [Xenopus] – 28% Rtf1 protein [Mus musculus] – 27%

				CTGTCCCTGTCTGCAGCCCCCCCCCGTCAC TCTGCTCACTGGAANNNGTCCAGCTCNN CTCCGGNNCCGCCGGCCTCCTGTCGGGTC TGGNGGGACAGGCNGAACCGATTAAGAT CCTCACATGCTNCGTTGCGTAGGAACNGC NGCTGNTNCTNNGGGGGNCCGGNNNNN ANGNANNCGANNGAAGAAACCGCCCCNG NANNTAAAATCCNNTCTAAANTTGNAN NGNCCNNGGAAGCGGGNGGGGGCGGNNN ACTCTGCNTANACTGCNNAACCGNTGNN TAATTGNNNTTANNGGNGGTTANGTTNT TNCCCGCNCAATNCNAACAANGANNGCA CTTTNNNNCTAANGACCCCCACGANATT TNCNGCTTGCANTNATATACGGGGNAGG NNGATTTNNNNGNNACAGGCCGCCTCTC TAGGAACACCGTTTGGCCCCCCGACTCNC CAGTTGTTAAGTCCCNCNCCNGATGNTT TCTTAGTAGTTTCCCGTNTANNGTNNCA NGNCTCNNANNNNGGATGATAANNNNN N			
DR10-3a BE12	UP	Taq	Seems normal	>dr103abed12UPTaq NNNNNNNNNNNNNNCCNNNTANNNN NNNGNGANCACAAGNTCGAGTGNTTTGT GTNGAANNGGGAGGANNTNNNNNNNN NNNNNNNNGCGCNCNNTNTNNNGAGGA TTACGCNCCCNCGNNNNNNNNGNCANA CTANCTGCCGGAAAGACTCCATTCTN CANTCTTCANTTCATCATCCTACTTNTCT CCTNTTTTNTCNNGTTGTNNGCTGANCC TGCATTTGGNNAACCCTTCGGAG ATCANCCNNCTGCCGCGGGTGNTGTCGCC NTACTGTATCNTCCCCNNCCTTCCNNTGT ANTTGTTTNCTTNCCACCTN	No significant similarity	No hits	
DR10-3a BH10	UP	Taq	Phototaxis away from light, swim slower	>dr103abh10UPTaq NNNNNNAANNNANNNNCCNTTAGCGGA NTGCGATCACAAGCTCGAGTGGTCTGTGT AGAAGTGGTAGTGATCTNNNNNNN NNNAANNNTGCTGNNANTAGNNATCAA NNNNCNANCCNNNNNANNNTTGANNTN CNCTGANNNNCCCNNANCCANNTATT ANNCANNNCCTTCNCCNCTCCANCTTCT NNTATNTATNCNTGTCTCNNTTTGNTCA TCCCANNGAAGACNACTCNNCAGC CCCCGGTCCCGTCANTGTGTGGAATCCCC TCCCTNCNCTGANATANCTGCNCGGCCCA CTCTTCGN	No significant similarity	B-tubulin	
DR10-3b AB9	DP	Taq	Phototaxis, can't swim straight	>dr103bab9DPTaq NNNNNNNNNANNNNNNNNNNNN NNTANCGNANTGCGATCACAAGCTCGAG TGGCCTGTGTAGAAGTGGTAGTGATCTAG NTGNCNNAANNTGGCTTTGGTAGCTCGC TATAATGCCTTTGCAGTCGGGGGCCTGGN TATTTAAACAGCGTTCGCCCTGCCNCGGC ATCGTANCGCCCATGCCGCCCCGAAGGAG CTTCGGGGGGTCGAAGCATCNTCGGTGTT GCATGCTGCGCCGGGAANCCGTCTCGCAG CCCGCCCGATGTTTTGGTGGATGTGTCAT ANCACANAAAGAATCTTGACCNCGAATT CNNAGTCATGGNCACGGTTGGTTCTTGG GTGAAATTTGTNNCCCCTCGNGNNNTTG GNGGTGTCNGAGNNNGAAGCACTGNCNN GANCNNNGGGGTGCCAATTGANNGANCT NACTCNNATNCAATGCGGTGNAGTCAGG AAGCGCGCAANNTNNATTAAANGACNAA GGTTTNNTGAACNAATGNATGGTTCTTT GTTTCGCAAGGNNAGGTTNGNNAAGAGG CCGACCCANNNTTCNCGCGTTTNTGATTC TNCGNNATCNNTNNTGGACACTTCCTTG TCNTCNCTTTTCTTNGGTNTGNGCTCCGC NGTNNTTNNNNNNNNNTCTTANTCGCC TGCNNTCGNNCGCTGCTCTCCATTTTTTT TTTTTANNTTACCCGAANGTCTTTTCCAG CGACNNGCTCCNNGNNNCNNCTNNTGCC NNNNTNTTTTTNNATANTNCTNNNANN AGAANNACNCANCNNANNNNAG	B-tubulin	Beta-tubulin	
DR10-3b AB9	UP	Pst	Phototaxis, can't swim straight	>dr103bab9UPPst NNNNNNNNAANNNNGNNNNNNNTNNG CNNAGTGNGANCACAGGCTCGAGTGGCC	No significant similarity	Beta-tubulin	

				TGTGTANAAGTGGTAGTGATCTAGGNGN NNNGTNNGGCTGGGGAGCTCGNTGNNGG CNTTTGNANCGGTTGNCTGGNTANGNNA GNNNNNNTNCCTGNTGTGGNGGNNNTC GNNGNGTNNCGTGGGGGGGGCNANNNG ATTGGAATCANCCTTNGNGNTTTGTGTA CNTNGGNNGNATTCTCCNTTTTNGNTAA TNTGNGCNCNCGNTCCNGGGAGGGNCNG NNGTCTTANTGAGTTNNCCTGNCNTGAN NAGGGTNTGGGGTTGGGGTTGNTTCGGT GGTTGNTTATCCCCNGGCGGAAGCNCTCC AAGCTTCNANTTGCCCCNNTGNGNNACA GGATATGCCTTTCCANTGGNNNGANGCG NNGNNTNGTTGTCAGGATGACTGGAGAC GTGAATCCTTTGANNNAAGTACCCCNTC CCGCNAGNGTNGCNGTGGNCCCNGGANA NNGGGATCNNGACTGCGCNNNATNGTNA NNTCNTGTTTTTTNTTNCTANAACTAAG TANTACAGACTTATCCGTCGCCTTGTTAT TCTCTGAGANAAAGAGTAGAAGNAGNCN GGAACNNNANNATGANCACGNTGNCTA NAGGCGGAGTGCTANTTATGATATATTT TTTTTGTGAAGTNGAANNGCATANNAAA AAAAAACAANNNTGTGANANTNGCNAT GCCGTATGTAGTTNNNNNNAGCTAGCTC ACNTTGAAACACATCCNCAANNACANAN NTCNNANNNANTANNAANNNGNNGAAG AGGNTATNTTTTTTTTATGGGNGNTGGC GNACNNACCCCNCCCCCANNNCNGAAAA ANAAAANACNCGTTNNGNGATAGANAN AGGTNNTNNNNTATNTANANNNCNCCC CCCCCCGNNNNNNANNNNNNGNNNNNN NANATATNTNANCCTNNNTGCNNGGNC NANNNNNNNTNCTNTCCNTNCNNNNNN TGGTTNTNTNNTANTANTNNTTNNTNA NNANNNNANAAGNAAGGGANNNNNGN NNNNNTTNGNTNTNNNTTGGGTGNNNN NCNNNNNNNCCCCNCCNNNNNNNNNN NNNNNTCCNNNNCNCCNNNNGNNCCGG GGNGGGGGGGGNNGGTAANNNNNNTNNN AANNNAAANNANNNAAATNNNNANNN NANNNNNNNNNN			
DR10-3b AB12	DP	Alu	Slow, not many cells swimming, can't swim straight, many oblong-shaped at bottom	>dr103bab12DPAlu CNNNNNNNNNNNCNTGNNNGACGACG GCGGTGGATGGAAGATACTGCTCTCAAGT GCTGAAGCGGTAGCTTAGCTCCCCGTTTC GTGCTGATCAGTCTTTTTCAACACGTAAA AAGCGGAGGAGTTTTGCAATTTTGTTGGT TGTAACGATCCTCCGTTGATTTTGGCCTC TTTCTCCATGGGCGGGCTGGGCGTATTTG AAGCGGGTACCGGGCCCGTCATCCCATGG AAGCTGTGTTGATGATAGGATAGCTTAA GGGTGTTAACATAGAACAAGTCCCTCAGA GGCAGGGACGACAGTAGTGCTTGCGTAGC GAGTAGGTGCTGTGTTGTATTGGTTGCTT TAATGGTTCATAGACTTCATGAGATAAG AAAGCGGTGCGGGGGCGGGGCCTATTTAT ACGGGCGTTTCAGGAAATTGGTCAAGTGC CATGCCTGGAGTGAGACCTGGAGCATCGC CTACCGCCGATACAGCGCTACATGCTACA ATATTGGATGTTTATGCATCCATGGGCGA GCGGGCGCTCATTTCGTGACCTAGGCGGA GCGCGATACCATAGTTTGTCAGGAATGCA ATGCCGAGACCTACAGTGCGGGGTGACTT GATTAACCGTTATGAGTGCAACGAGACTG GGCGACTGGCATTATAACAACCGGATGTA TTCCGGAACTGACCGCGACGGCTGCGTCA AAAAGTCCGCGGTCCTGTGTTACGTCCGC GGCCCCCCCCCGTCACTCTGCTCACTGGAC CGCGGCNNCCCCCCGTCACTCTGCTCACT GGAANNCGNNNNCNANCNNNANNNNN NNCGCTGGNANANANNNNNNGAAGTGN NNNATCCCGGACGAAGTGGNNCNNNNN NNCNNGCGNNCTCCNNNNNANCGCCCN NGNCNCNGANTAAGGATCCCGCNNCGNN GANAAATNNAGNNCCTNNTTTAATCATN ACCTTNNCCNNTNACAAACGNNNNTTN NNGNAANANCTGNTNCNNANNGNNNNA	C. reinhardtii pyruvate-formate lyase (PFL1) mRNA (100%) Pfl gene Rubisco	mid-chr. 1 Pyruvate-formate lyase Formate acetyltransferase rubisco	Formate acetyltransferase - highly conserved in bacteria

				NNNGTTNNNTTNNNNNNACNNNNCNN NNNNNTNNNTNNTNTTTNANNNGTANA NNNNGTNNNNNNNNCANNTTTNNTTN NNNNNNNNNNNNCNNNATTTTNNNNNNN NNNNCNNGGNNGNNNNNNNNTNNNTT NAANNNNNNNCNNNNNCNNNNCNNGN NNNNNTNNNNTNNNNNNNNNNNNNAN N			
DR10-3b AB12	UP	Pst	Slow, not many cells swimming, can't swim straight, many oblong-shaped at bottom	>dr103bab12UPPst NNNNNNNNNNNNCCCCNTAGCGGANTG CGATCACAAGCTCGAGTGGCCTGTGTAGA AGTGGTAGTGATCTAGCCCGGACATGCGA TTCGGACCTTGTCATCGTCTGTACTGTCC GTGACTTGTTTACCTCAGGCCGTCTTTGA CGCTTGGAAACGTTCGTGGCATGCGCCTG GGTGTTCCGGAGCTGGCCTTCAGGCAAGT TCGCAGTGATTGGGTAACCCAACCTTTCG GCACGCCTGCATGCCAGTCGGGCGTTGCC GCATTTACTGGTTAAGCCTAACGCTTTTG ATGCTACCTATGTAACCCAGGTCGCCGAT GCGCTCGATGGCCGCTGCCAGCGCGGCCG CTGAGGCCCTCCCGGTGGCACCCAGCCAC AGCTGCGCTGCTGACCCCGACAAGCACCC GCACCTGCCCGACCCCCGCCCGAAGCCGGC CGTGGACGCGGGCATCAACGTCCAGAAGT ATGTGCAGGTGCGCACTAACAGTGTCAGG GTGCCGACAACGTTGTGCAGTGCCGGTTT TGTGGCGCGGTGGGGCCAGGTCGAGAGCG TACGGACGGGGTTGGTTTGCTCGCTGCCA CGGGCTCTGTTGTGAATCATGGATTGCCT AGAACCACCATCTGACGCCTCTTCCGCCC ACTTCCACAATTCGCGCCTTGCACAGGAC AACTACACCGCTTACGCCGGCAACTCGTC CTTCCTGGCTGGCCCCACTGACAACACCA AGAAGCTGTGGAGCGAGCTGGAGAAGAT GATTGCCACCGAGGTGAGCCATGCACATC GACGCCTCGTGAGGTGCTGGCAGCAGCCC AAGAAGTGCGGATTTCNNTGTCATGCAT ATGTATGCGACAAGCTAACTTCAGGGCCT CACAACAGCAGCATCCCGGGCAGGGCCCC ACCACAACCATGGACCGCGGCNNCCCCCN NNNNNNNNNNNNNNNNNNGGA	B-tubulin Pfl (pyruvate formate-lyase) gene PFL1 mRNA (100%)	Chr. 1 Formate acetyltransferase Pyruvate formate-lyase Chr. 12 B-tubulin	Formate acetyltransferase Pyruvate formate-lyase [C. reinhardtii]
DR10-3b AF8	DP	Alu	Can't swim straight	>dr103baf8DPAlu NNNNNNNNNNNNNNNNNNNNNNNNN NNNNANANGNNGGAAANCTCNAATGNC CTGTGTAGAAGTGGAAGTGATCAACNNG NTTNANNNGGCATTGNAGCTCGTCATCA TGTGTTCTGTACCTGACTTNTTTTTATCT NNACNTCTTTCACCCTGGNANCGTTCGN GNCNCCCNCCTGGCTGTTCCNGAGCTNN NCTTNTTGCAANTTCGTAGTGATTGGGT GCCCNNNNTNTNGCNNNCCNGCGTGCCC CNACGGCTCANNCNCCCTTGCTGGTTAT NCCATANNCTTTTGCNNTACTCATGAAA CCCNNGTTACNNATGCGATCGATAGCCCC TGCCAATNNTNCTGCTGANGGCCTCCCGG TGTNNCNCTATCNNANCNGCNCANCNNA ACNCTNTAANNNCNCCCACCTGCCTNAA CCCCNCACCAACACGGACGTGGCNNCCGC ANTCAATNNCANGAANTATNTGAAANN GAGCAGAACANCATCANNATNGGNNANA NACANTGNNNN	no significant similarity	No hits	
DR10-3b AF8	DP	Sac	Can't swim straight	>dr103baf8DPSac NNNNNNNNNNNNNNNNNNNNNCNCCN CNNNNNNANNGNNANNCNNNNGCTCTT GGTTTGNGTAGAAGTGGTCTTGATCTNN GAGNNNAANGNNNGCTTTGGTAGATCGC TATAATGTCTTTGCAATCGGGGGTCTGGT TATTTAAACAGAGCTCCCCCTGGAGCGGC NTCGGAGCGCCCATGCAGCCCCTAAGGAG CTTCTGGGGGTCGAAGCATCATCGGTGTT GCATGCAGCGCCGGGAAGCCGTCTCGCAN CCCGCCCTACCTTTTGCTGGAANTGTCAT ANCGCAAGAAAGAAGCTTCCATGGGATG ACGGGCCCGGTACCCNCTTCANATACNCC NANCCCGCCCATGGAGAAAGAGGCCAAT ATCAACGGAGGATCNTTACAACCAACAAA NTTNNNAAACTCCTCCGCTTTTTACGTGT	B-tubulin	Chromosome 2 Rubisco	

				NGAAAAAGACTGGGTACGGNGAACGANG GTACCGGNNNCCNTGNCCCCTCCATGCN			
DR10-3b AF8	UP	Alu	Can't swim straight	>dr103baf8UPAlu CNNNNNNNNNNGGGNNNNNNNNNNNN NGNGNGNGTGGNTGGNNGAACNGNTCT GTGTGAAGAGGGGTGATCTAGGCNNGNT TCTNGCTGAGNAGCTTTTANACGCCGAN NNTGNGGAGGNATGGNTNTNGTGNNTT GNTCGGNGAACCGNTGNGTTTTNNCCCC GTGCNNGCTGATNNNNNTTTNNGGGTG NNTNANAATCNNAGGAGTTTNNCGCTTT TGTGGGATGCCGCCACCCNCCNTGGAGAT GGGCCGGTNTCTACGTGGGNCGGCTGNA TGTNTTTGAGGCACGGNCCCGGNGCTGGC TANCNTGNTAGGGGCTNNTGNCNANTCG CTNTTTTCNNTATCAAGAGGAACTGGCG CGTTCCTCTGGGGGCCGCCGGACACCGCC CCCGGCCCNGANNNNNATCCNTCNGGNN NNNGNANGCNTCGNGATNGNCTNGNCN GNANAANGCGTNGAAGGNNNNCNGNNN ANNGNNAACGTANTTACTCCCNTTTNNG NNNGCATCTTTTNNNCANNGAAAGGGGA GGAATTTTGCATTTTNTTGGTGTNNACN ATCTCCGTTGATTTTTGCCTCTTTCTCNT NNGCCGGCTNGGCGTANTTTAAAGNNGN NNNNNGCCCNNTCCCNTGGGAANCTNAC CCCCCCNCCCTTTTNTNGGNTNGNNAAN	S. hygroscopicus hyg gene	No hits	
DR10-3b AF8	UP	Sac	Can't swim straight	>Dr103baf8UPSac NNNNNNNNNNGNGCCCNTTAGCGGAGT GCGATCACAAGCTCGAGTGGCCTGTGTAG AAGTGGTAGTGATCTAGGTGTTTGAATA TGGCTTTGGTAGCTCGCTATAATGTCTTT GCAATCGGGGGCCTGGCTATTTAAACAGC GCTCGCCCTGGAGCGGCATCGGAGCGCCC ATGCAGCCCCGAAGGAGCTTCGGGGGGTC GAAGCATCATCGGTGTTGCATGCAGCGCC GGGAAGCCGTCTCGCAGCCCGCCCTACCT TTTGCTGGAAGTGTCATAGCGCAAGAAAG AAGCTTCCATGGGATGACGGGCCCGGTAC CCGCTTCAAATACGCCCAGCCCGCCCATG GAGAAAGAGGCCAAAATCAACGGAGGAT CGTTACAACCAACAAAATTGCAAAACTCC TCCGCTTTTTACGTGTTGAAAAAGACTGA TCAGCACGAAACGGGGAGCTAAGCTACCG CTTCAGCACTTGAGAGCAGTATCTTCCAT CCACCGCCGTTCGTCAGGGGGCAAGGCTC AGATCAACGAGCGCCTCCATTTACACGGA GCGGGGATCCTTATCAGGCGCCGGGGGCG GTGTCCGGCGGCCCCCAGAGGAACTGCGC CAGTTCCTCCGGATCGGTGAAGCCGGAGA GATCCAGCGN	B-tubulin (14-287) Rubisco (318-550)	B-tubulin (chr. 12) Rubisco (chr. 2)	
DR10-3b AG7	DP	Taq	Swim slow, not straight	DR10-3bAG7DPTaq NNNNNNNNNNNNNNNNNNNNNNNGCC CCCTGNNNNNNGGCGGTGGATGGAAGAT ACTGCTCTCAAGTGCTGAAGCGGTAGCTT AGCTCCCCGTTTCGTGCTGATCAGTCCCT GCGGCATTAGTAATGAAGGTGTGTGCATA AGCGTCAAGCCGTTGAGGCCGTTGCGGAA TAGAAGAGGTTTGTGCGCTCGGACCCCGT AACAGCTGCTAGGACTGCCGCCGTGCAGG CGCAATTTGGCGGCGGACAACGGCCATAC CAAGCAGCACACGCGCACGGTCCACCCAG CCCGACAGCACTCGGCTCAAACAGGTGGG TGCCTACGTACCGGGAAGTGGTCCGCGAG AATGCGCAGCGTGGCCATAGACACGCCTA CATGAGCCATGTTGCGGCGCAGCAAGGGG CAATCCATAAAGTGTTATGCGGCGCGGTT TTATATTTCTGATGCAATGGGCTGGCGCA CATGAGCACACCACGTTGCCGCAAGTCAC AGGCAGTCCTGTTGCTGCGGCTGAGTGCT GACTCACCCAGCGGCGGCGAGTTGGCACG TGAGTAGCCGCGCATGTCCATCAGCCACA CCCTGATAGTTCACACACAGGCATGAGGA TTACTAACTGCGTGCAAGGACTCCTCGCC CTTCCTATCCCCTGCTCCTACCTGCTCTCT CAAGCCCTCCTTCCTCTTCTCCGGCCCGTA TCCGTTCGACTCCNCCCCCCGTCACTCTGC TCACTGGANNNNANNCCCCCCCCNTNGT	Rubisco	Rubisco	Phosphatidyl transfer protein

				TTTTTTTTTNNNTTNNATGAANANNTNA AANNCNTNANNNNNNNNNNNNNNTC GNNNNANNNANNAATNANNNATCCCNN AGGAACTGGCGCANTTNCTCTGGGGGCCG CCGNAANNCGCNCCCGGNNCNGNATAAA GGATCCNGNNNNNGNAATGGNACGCGCT NNTTNANCTGAGCNTGCCCCTGANNAAC GNNGTGGNAGGGAANANNNGCNNNANN NNCTGAANNNNTANNTTANCNCCCGTT NNNGCNGANNNNNNNNNNGNNTTNNN NANGNANNNGNNGNNCNNNNNCGTCN NNNNNNAGAGCGTTNNNNNATAAAAGA NNNNNNNGNNNCNNNCCNNNACNNNN NNNNNNGNNCNNANNCNNNNNNNTTT NNNNGNNNAANNNCNNNNNNNNNNAN NGNCANNNNNNTNNNNNNNNNANGNN NNANNCAGNANN			
DR10-3b AG7	UP	Alu	Swim slow, not straight	NNNNNNNNNNNNNNNNCTTAGCGGAGT GCGATCACAAGCTCGAGTGGCCTGTGTAG AAGTGGTAGTGATCTAGNNNNNANNAN NNNGTTTTGGTANCTCGCATANNGTCTT NNNGANNNNNNNGTGGTNTTTTAGNGC GTCANCCCNNNNGCCATCNNACNTNCAN CCNNAGGGGGTTTTTTACTATTAAAANA NAACCGGAGTTCTTGCTTTNAGNACANT ACNCNTCATTCAGACCANGNNATGNTTA CCGCTTCANACCATACCTTTNACATANAN GCTCCNCTNTCAAAGACNGGAAANCATT CTATCTTCGGTTNCANTNNNTACTTNNT GTTCTCCNGGNTCNN	B-tubulin	B-tubulin	
DR10-3b BB8	DP	Pst	Can't swim straight, slightly slow, possible that cells may swim to bottom of well after a couple of minutes	NNNNNNNNNNNNGNNNNCNNTNTCGC NGANNGCGNTCACGNNNNCGAGTGNNN TNNGTAGAAGTGGTAGTGATCNAGNNNN GNNAGGNNGNGGGNANCNACTTGTCGGC CGAAAAGATGTTTTTGTGCTCCTCTTNTT GNTCGANAGGCTGCTGTGGTGACTTTCN GAGGGGNACCATGGGGGGGGTTTTTTGT TGTNNATTCCTCCAGGATTTGGTNNNCN GGNCANCTNCTTCNATTTNCNTTTNNTC TGNTNGCGCTNCGNAANTTGTGTGTGCN CTGNACTNATTCTCCTNNATGACGAAGG GCCTGGGGCTGGTGCGCTTTCGGTTGTTG TTGNTCTCANGGCNNNGNCNCTGNCAAN CTTGANTNCTNGGCTTGGNAGNNGGATT GTCNANNGCAGTGGANNGATGCGCAGCT AAGTTGAGGTGAGGAGCGCAAACNTGAA ATGGCGANTGNGTGTTTNCNNNCGTCTA GGNTTCCTTTGTTCCCAAGGACAACNGGA CNCTGANTCTGNCTNTTGCCCGCAACTTT TCNTNNNCCNAANNNTCTNNCTANNAT TCCTCTTCGTCTTTTTGNTGATTCTCNTA GNNCTNAGGGTGGGTCTGNGGCACCACN TGAGCACCTTGNTGTCAGGCNNGTCCAGT CCTATGATCTTCTTTTTGNATNAGANGA NNGNNCTNNGANCANCAACCCTNCCNNG ACNANCGCNAGGCNTANGTANTTTATGA NGTAGCTNNNNACGANNNNCNNANNNT ANNAACGGCTCGGNNNNNNNACNTNNN NGGNNACNNCGNTCTGGTACCTGGNNNN NCNNNCTATGANTTNNNCNTNANANNG AANANGNTNCNGCTNNCTCGGNANNNA NNNANNATGANNNACNNNNCNNNANGC CNNNANNNNNNNCNNAGTACTNNCCTG GAATANGAGCCCNANNNNNCNCGNCNN GAANCANGNNNNNNANGAAGATGTTAN ANTANANNNCNNNNANNACNNNCNNN NNNANNTGNANTNANNNNNNGAGTACT TCNNNNTTNNNNCNGAGNNNANCTNCN	No significant similarity found	No significant similarity found	
DR10-3b BB10	DP	Alu	Seem to swim to bottom of well after couple minutes	NNNNNNNNNNNNNNNNNNTNNNNNN NGACGACGGCGGTGGATGGAAGANACTG CTCTCAAGTGCTGAAGCGGTAGCTTAGCT CCCCGTTTCGTGCTGATCAGTCTTTTTCA ACACGTAAAAAGCGGAGGAGTTTTGCAA TTTTGTTGGTTGTAACGATCCTCCGTTGA TTTTGGCCTCTTTCTCCATGGGCGGGCTG GGCGTATTTGAAGCGGGTACCGGGCCCGT CATCCCGTGGACGGCCCGGTCCCAGGGCG AGCGCTGTTTAAATAGCCAGGCCCCCGAT	Vector	Rubisco B-tubulin	

				TGCAAAGACATTATAGCGAGCTACCAAAG CCATATTCAAACACCTAGATCACTACCAC TTCTACACAGGCCACTCGAGCTTGTGATC GCACTCCGCTAAGGGGGCGCCTCTTCCTC TTCGTTTCAGTCACAACCCGCAAACATGA CACAAGAATCCCTGTTACTTCTCGACCGT ATTGATTCGGATGATTCCTACGCGAGCCT GCGGAACGACCAGGAATTCTGGGAGGTGA GTCGACGAGCAAGCCCGGCGGATCAGGCA GCGTGCTTGCAGATTTGACTTGCAACGCC CGCATTGTGTCGACGAAGGCTTTTGGCTC CTCTGTCGCTGTCTCAAGCAGCATCTAAC CCTGCGTCGCCGTTTCCATTTGCAGCCGC TGGCCCGCCGAGCCCTGGAGGAGCTCGGG CTGCCGGTGCCGCCGGTGCTGCGGGTGCC CGGCGAGAGCACCAACCCCGTACTGGTCG GCGAGCCCGGCCCGGTGATCAAGCTGACC CCCCCCCGTCACTCTGCCTCACTGGN			
DR10-3b BB10	UP	Pst	Seem to swim to bottom of well after couple minutes	NNNNNNNNNNNNGNNNNNNNNTAGC GGANTGCGATCACAAGCTCGAGTGGCCTG TGTAGAAGTGGTAGTGATCTAGGTGTTT GAATATGGCTTTGGTAGCTCGCTATAATG TCTTTGCAATCGGGGGCCTGGCTATTTAA ACAGCGCTCGCCCTGGAGCGGCATCGGAG CGCCCATGCAGCCCCGAAGGAGCTTCGGG GGGTCGAAGCATCATCGGTGTTGCATGCA GCGCCGGGAAGCCGTCTCGCAGCCCGCCG ACGGCCCGGGTCGCCCGGTGGACTAGTTG ATTCCGAGCCGGCACCATTCTCACGGCGG CGCCATCATGTGTTGGTGGCACGCCACGC TGATTTCACCGCTGGCGCGCGCACGCTGT GCCGACAGGTGCTACTTGCATTGCATATG AGGCCTTGAACACCTCTCAAATATTACAA CGCGGCGGACGCCGTTGGCTTGCCTGCGT CCAGCAACGGCACAGCAGACCAGACGCGG ACGCGGCACTGCTCAACGGCGCCCACAGC TTCTGTCCACCTCGACCGTTCCCGTTGCT TGAAGGCCTTTGCTCCGATCCATCGACTT GGTCACCTAGCTTGAGACCACTATCCACT CTGCCTCTTCGGCAAGCCTGGCCGCAATT CGAATAAGTTCCCACCCGCGGTGCTTCCA CGATACACTCCTGCCACGATGACACCCGT AACACAAGACGGCACCGACGTGACGCTTC TTCTTGACTCGTCCCTGTCTCGTGTCTTT GTCTCTAGCTCTATCGCTACACCTACTTG CGCAATCTGCTTCGTGTCCCTGGCACAAG TAGCTGCGCGGGTGTCACGCGGGTCCGGA CAACACACACTCACAGCGAAACGTCAACT CCACAGCGCCCGCTCCCACATGGCCCGCGC GGCGTCACATAACGTTTACCACCCGTCNN ACATACNGTACGTAGCATGGNCAAAACC TATATTCCNACANCGGTNNAAGCACACT TGANGGACGGGTAAAACTACCTANTACA TGGNAGGTTGGTCAATGCAAATGGTGTG TCGGNNNGCGACCACNTGTTCNTCNGNA TCCCACANNCGCAAGGACGTACNGNANC TTNANCNGGCANNCTGNCACCTGGNNAA CTTGNANCCGCTGNNNNNNNCTGGNNN CNNCNNANNANNACTNGNCCCGGNNNN NNNNCNTNNNNNNNNANCNNNNN	Eukaryotic release factor 1 (ERF1) mRNA B-tubulin	Chr. 13 Peptide chain release factor eRF/aRF subunit 1 Chr. 12 B-tubulin	
DR10-3b BC2	DP	Alu	Can't swim straight	NNNNNNNNNNNNNNNNNNNNNTGAC GAACGGCGGTGGATGGAAGATACTGCTCT CAAGTGCTGAAGCGGTAGCTTAGCTCCCC GTTTCGTGCTGATCAGTCTTTTTCAACAC GTAAAAAGCGGAGGAGTTTTGCAATTTT GTTGGTTGTAACGATCCTCCGTTGATTTT GGCCTCTTTCTCCATGGGCGGGCTGGGCG TATTTGAAGCGGGTACCGGGCCCGTCATC CCAGGGCCGCACCGGCCGACGCTGACTTG ATGGGGTTGGCGCCAGTCCGCGGCGCGAC GACATGCCTTGACGCGTCGGCAGCCACCT GCAGCGGCTGTGGCTGTGGCTGCGAGCTG GGGTCCCCGGCGTGCCTGGCGGCCAGTGC CTCATCCACCAGCCGCAGCACCGCGGCCT TCACCACTTGGGAGCGAACCTGGGCGGGA CAGGAGGAAGAGGCAGGAGGATGGGGTT AGCCGTTCACGGAGAACGGGGGAGAAAAC GGTCAGCCGCACCAGCCTCACGCACTGCA	Multi-eye (MLT1) protein Rubisco	Chr. 12 Om/DAP/ Arg decarboxylase 2 Chr. 2 rubisco	

				CAAAGGCTCAAAGGACCATACACGTCGTT CATGCATCAGACCCATCCAGCAGCGCCAG TGCCGGCCCACGCTTGCATCCTCAATCTC TGTGTCGGTTGCCGTTGGCTCGTCACCCC CGTCAGCCCGCACCTGGCCGTACTGCGGC ATGGCCTGCACGCGGCCGTCGATGTAGCC GCTGGCGTACCCGCGAATGGCCTCCAGGC TGGCGGCGTCCAGGCGGCTGAGCTGCGCC GTCAGCGCCGCCGTGTCCAGCACCGCGGA NGGCCCCGGCGTCAGCGCGCTGCCGCCCA CACCACCGCCACCGCTGGCGCCTATAAAG CATACCGTTCATGGGCACAGGGTGCAGTA NGGTTGGGTGGAGAACAAGGAGCACATA ACAGCAATGCGAGGCGCTGCGGCCTTACC GTTATCGCTCATTGACGAGCTCCGGGTGT GCTGCAACGCCGTGNNCACAACTGCCGCC GCAGCCGCCGACAGANCGGAAGCTGNACC CCCCCCCGTCACTCTGCTCACTGGAANNT NACCCCCCCCGTCNNNNNNNNNTCANTG GAANNCACNNCNNNNCCCTNNNNNNNN NNNNNNNNNNNNNNNNGNNNNNNNN NNANNNNNNNNNNANNNNNNNCNNTN NNNNNNGGGNGACNNNNNGNNAAANN NNNANCNNNCNNNNNNNNNTNNNTNA GNNNNNNNNNNNNN			
DR10-3b BC2	DP	Pst	Can't swim straight	CNNNNNNNNNNNNTNNNNNGACGAAC GGCGGTGGATGGAAGATACTGCTCTCAAG TGCTGAAGCGGTAGCTTAGCTCCCCGTTT CGTGCTGATCAGTCTTTTTCAACACGTAA AAAGCGGAGGAGTTTTGCAATTTTGTTGG TTGTAACGATCCTCCGTTGATTTTGGCCT CTTTCTCCATGGGCGGGCTGGGCGTATTT GAAGCGGGTACCGGGCCCGTCATCCCAGG GCCGCACCGGCCGACGCTGACTTGATGGG GTTGGCGCCAGTCCGCGGCGCGACGACAT GCCTTGACGCGTCGGCAGCCATCTGCAGC CCCCCCCCGTCACTCTGCTCACTGGAGTT GCTTNCNCGTTCNNGGNNCTCNNCANCC CCAAAAGNGCTGACCCCCNCGCCTCCNAA TCTTTNANNCCCNANCNNCCCACNNNNN NNNCGNGTTNNCCTGNGNTTNGGGNGCC TTNTTCGGNTTGTGATGTGCNTCTGACG ACNGACTANTGNNTNNNNNCCCTCCANG NNNGANCCNGCCNNNANNCCCNTGATTC AGGTGAAANN	Multi-eye (MLT1) protein Rubisco	Chr. 2 Rubisco Chr. 12 Om/DAP/ Arg decarboxylase 2	
DR10-3b BC2	UP	Alu	Can't swim straight	NNNNNNNNNNNNNNNTAGCGGAGTGCG ATCACAAGCTCNNGTGGGNCTNGTGTAG AAGTGGTAGTGATCTAGGTGTTTGAATA TGGCTTTGGTAGCTCGCTATAATGTCTTT GCAATCGGGGGCCTGGCTATTTAAACAGC GCTCGCCCTGGAGCGGCATCGGAGCGCCC ATGCAGCCCCGAAGGAGCTTCGGGGGGTC GAAGCATCATCGGTGTTGCATGCAGCGCC GGGAAGCCGTCTCGCAGCCCGCCCTACCT TTTGCTGGAAGTGTCATAGCGCAAGAAAG AAGCTGACCCCCCCCCGTCACTCTGCTCAC TGGANCGAANTTGNNANNNNNNGTNNC NNTNCCCGCGCCCGGTNCNGCCNCTCCN NNCTTTGCGTGCCCTNNTCNGTTACGTT TATTCCTTTCTCACTNTGNTNNNANAAC NACCNGNGTAGNNGNNACATCNGCNAAG NTNAACATTTGTTTGAGGTTNCNNAGCN CCCNTNNGNTNTTNGNNNTNCNNGNAA NNNNNGNCACATTNCNACNAAATTCCCC NATTNNTCTTCNTCNNNANTNCTTTANA CCCCCTTTTTNNNCTCTTTCNAGATCGTN TAAGAGAAATNGNNNNNCGNNTNTNNG AGNNNNATAAATCNNTTGTCTTCTAGAG TCTGCTCTTCNA	B-tubulin	Chr. 12 B-tubulin	
DR10-3b BC2	UP	Pst	Can't swim straight	NNNNNNNNNNNNNNNNNNNNTAGCGG ANTGCGATCACAAGCTCGAGTGGCCTGTG TAGAAGTGGTAGTGATCTAGGTGTTTGA ATATGGCTTTGGTAGCTCGCTATAATGTC TTTGCAATCGGGGGCCTGGCTATTTAAAC AGCGCTCGCCCTGGAGCGGCATCGGAGCG CCCATGCAGCCCCGAAGGAGCTTCGGGGG GTCGAAGCATCATCGGTGTTGCATGCAGC GCCGGGAAGCCGTCTCGCAGCCCGCCCTA	Multi-eye (MLT1) protein B-tubulin	Chr. 12 Om/DAP/ Arg decarboxylase 2 Chr. 12 B-tubulin	

				CCTTTTGCTGGAAGTGTCATAGCGCAAGA AAGAAGCTGCCCACAACCGTGCCGGCGCC GGCCAACCGCGCCAGTCCTGGCGAGGAGC GCCTGCCTGCAGTGGACCTGGACCTTGAC CAGGTGTGGAGCGCTGATGGGAACAACTG TCCTGCTGGTGGCGGCCGGTGGTTAATTA CGGCATGCACGCAAACCTTACAGCCGTGC GACACGATCGGTGTCTTTTGGGTTTCTTA CGGCCTTGCGGTACATCCTGACTCTCCTT CCTGCCCTGCCTGCGGTTGCCGCGTAGGC CGCGCGCTGTCTACCGCGCCACAGCCCCTC GGACGGGTCGTCCGCACCACTGCAGCCCC CCCCCGTCACTCNGCTCACTGGANTTTCT NNNACNNNNNTAANNCNANNTNNTGCA CNGCNNNNNNTCTCCNTTTTTTTTNTNN TNGNCGTCCNGCNCNNTCTTATCNCNNC N			
DR10-3c 1f11	UP	Alu	Swims to bottom of well after a couple of minutes in the light Shaky, can't swim straight	NNNNNNNNNNCNNNTAGCGGANTGCG ATCACAAGCTCGAGTGGCCTGTGTAGAAG TGGTAGTGATCTAGGTGNTTGAATATGG CTTTGGGAGCCCGCNGANTGACGTTGCGC CTCGGGGCCTGGNTAGTTAAANNTCGGT CGCCCTGGAGCGGTATCGGATCGCCCATG CAGNCNCGATGGGGCTACGGGGGGTNTA CNCTCAAACNGNNNGTGTGAACCCTGGN AGCCGCCCGTGGTGGTGGGTTTAACTATA AAGAGGATGATANTAGCGCTTCNTCGAG AGCNGGAANNNNTNNAACANTACCGAGT TGTTNTNCACGGAGAAGCCCCGATATCCC TANTCCACCAGTAAATGTGCTGCCGAACC NAGNACCTGGTCGCGCTTACNNNNNNNG CTCAGCCNTGNNNAGAGTGATCCCGGCG GCNGTCACANNCTNCGAANNNNAANATG NNNCNCGCCTTCTCCNTTNGGGTCTTTN NCTNNNNNGGAATNGNTGCTCTCGGNAT NNNTNTNNNGGCGACCNNNNNCAGCCC TCTCCCANTNNGGGTGTGATGCTGTNNN GGNNCTGCNNANNNNNCCTNNN	B-tubulin	Chr. 12 B-tubulin	
DR10-3c 2F4	UP	Sac	Swims slowly, shaky, not straight	NNNNNNNNNNNNCNNNNNNNTAGCGG ANTGCGATCACAAGCTCGAGTGGCCTGTG TAGAAGTGGTAGTGATCTAGGTGTTTGA ATATGGCTTTGGTAGCTCGCTATAATGTC TTTGCAATCGGGGGGCCTGGCTATTTAAAC AGCGCTCGCCCTGGAGCGGCATCGGAGCG CCCATGCAGCCCCGAAGGAGCTTCGGGGG GTCGAAGCATCATCGGTGTTGCATGCAGC GCCGGGAAGCCGTCTCGCAGCCCGCCCTA CCTTTTGCTGGAAGTGTCATAGCGCAAGA AAGAAACGCAAAACCACGGCAGCAAGAG GGCAGACAAGGACGAGCAACGGAGCCTTC AAACTTGCTTGCTGACTCCGACAAGCTGC CACCAGCTCTGGCGATGGGAAGCTGCAGC TCTAAAGCGCGTGAGCACTGGGTTCTTAT GTCGGCTTACTTTGCCACCGGGGCCTCTG CCTTTGAGCTGCAAGGACGGGGCCGTGGG TCTGCGAATCGCGACAAAACTCCCCAACA AAGCTTTGTAGCCTCGCTTGGATCTGTGA AATCCCCGTAAGTCGCGACCTTTCACTTG TGCTGCTCATCACCCACACACAGGCCGGC GGCGGCTGGCAGCGCTCGCCGCGCCCGTC CCCGCGCCCGTCGCCTGCCCCTGCGCGGCG GCCGCCCCCGCCCGCGCCCNANNCTACGC CCACCTCCGCACGTACNGCCATCCGCGGC CNCCCCCCGTCACTNNNNNNNNNNTGGN	B-tubulin	Chr. 12 B-tubulin	
DR10-3c 4a9	UP	Alu	Swims slowly, not straight, shaky	NNNNNNNNNNGCNCCTTAGCGGAGTGC GANCACAAGCTCGAGTGGCCTGTGTAGAA GTGGTAGTGATCTAGGTGTTTNNATATG GCTTTGGTAGCTCGNNATAATGTCTTTG NNATCGGGGGCCTGGNNNNNTAAACNN NGTTCGCCTTGGAGCGGCATCGGAGCGCC CATGCAGCCCCGAAGGANCTNCNGGGGN NNNANCNAANCAAGNCNNTAACCAGCCT GNNNNCNGNNGNGNNGNCTNTCTACCT ATTGATGTTNATNCNTATTNTTTCTCAN GCGNTATATGTAATTTNTNANTTCNGAA TTTTTNGTCANNCCATNNCCANGATATG CGTANTNNNCTNCTATTATTNCTCCCNA AGCTTGCTCTACNNCTCNCTTACCTCAAC	B-tubulin	Chr. 12 B-tubulin	

				TTNTCTGTCNTGCCANNNNTGANCNTAN NGCNTTCANNANTTNNANCNAAGACTT NGNNTCCGCTTTTTATNNGNTNNNTTCT TCTNGCTGGNCTNGNTTCTTCNNTTNCG GTT			
DR10-3c 4a9	UP	Sac	Swims slowly, not straight, shaky	NNNNNNNNNNCNNTTAGCGGANTGCG ATCACAAGCTCGAGTGGCCTGTGTAGAAG TGGTAGTGATCTAGGTGTTTGAATATGGC TTTGGTAGCTCGCTATAATGTCTTTGCAA TCGGGGGCCTGGCTATTTAAACAGCGCTC GCCCTGGAGCGGCATCGGAGCGCCCATGC AGCCCCGAAGGAGCTTCGGGGGGTCGAAG CATCATCGGTGTTGCATGCAGCGCCGGGA AGCCGTCTCGCAGCCCGCCCTACCTTTTG CTGGAAGTGTCATAGCGCATTCCGGCGCG TTCCTATGGCACATCAGTGGTTCGCAACG GTAGGCACAGTCAATGGTGCGCGAAGTGG GATGTGCCGCAGTTATGCGTCAGTTTGCG TGTGCGAATTGCATCTGCCCGCAAGAGTG TGTGGGCAGGAAGGCAAGGGCATATGAG CAGTCATCCAAGTACGCCATGCCTTCGCC CCAAGTTGATGCACCTCAGCACTCTGTAC AAATGTGTAGGTGCGCTGCGGCCCCAGCT ACTACGCACCCGCTCCTTCTTGTCCTCCTC AATGATGGTTGTGAGCTGTCGCCATTCCT CCTCGAAGCCCTGCTGCTCCTTGTCCGCCT GCGCCTTCAGGGCGTTCATCTCCCCAATG GCCTTCTCTCGCGCCTCAAATGCTCCATT GGGCCTGCTGAATCATGTCCGCCATGTCC CGCTTCAGCTTCNCCAGCTCCCGCTCCAG NTTGCTCTGGATGCTCTCAANATGATGCG CTCCNCCGNANGTNATTTATAGACTCCCG CAGCTGCNNNNNGCGTANNNNTNNNGN ANNTNNCGTACNNNNTCNANNNNNCTC NNCANCTNANGNNGCTTCNNCNNCNNN NGGNNNNNNC *this is 3' going to 5'	B-tubulin ODA1	Chr. 16 Chr. 12 B-tubulin	Flagellar outer dynein arm-docking complex protein 2 ODA-DC ODA1

B. Ca^{2+} ATPase Mutant Path Length Measurements

Path Length Measurements - BG8 in calcium

	Area	Mean	Min	Max	Length in pixels	Length in microns	Velocity (μm/s)	Average velocity per group
1	128	51.91	42.94	102.27	128.25	41.04	4.24	
2	206	49.03	42.00	58.36	206.39	66.04	6.83	
3	93	56.20	41.14	70.00	93.72	29.99	3.10	5.09
4	186	57.24	45.26	81.35	186.86	59.80	6.18	
1	150	48.92	34.00	65.99	150.84	48.27	4.99	
2	65	56.16	33.00	102.09	65.52	20.97	2.17	
3	65	57.77	39.23	82.55	65.90	21.09	2.18	
4	117	49.18	35.77	83.06	117.14	37.48	3.88	
5	41	60.02	45.00	71.30	41.49	13.28	1.37	
6	75	48.62	38.00	60.56	75.08	24.03	2.48	2.64
7	49	67.17	36.03	96.44	49.22	15.75	1.63	
8	125	46.09	37.00	54.39	125.90	40.29	4.17	
9	59	56.84	35.00	79.54	59.89	19.17	1.98	
10	50	53.44	37.49	66.99	50.43	16.14	1.67	
11	77	75.88	44.00	93.83	77.35	24.75	2.56	
1	91	49.14	21.28	75.62	91.24	29.20	3.02	
2	67	44.62	32.00	54.42	67.67	21.65	2.24	
3	97	45.25	38.08	56.81	97.79	31.29	3.24	
4	155	28.21	22.19	35.00	155.41	49.73	5.14	
5	84	68.81	32.00	97.67	84.06	26.90	2.78	3.15
6	73	60.76	32.62	89.19	73.20	23.42	2.42	
7	89	67.17	38.45	94.86	89.77	28.73	2.97	
8	102	45.07	35.00	58.54	102.57	32.82	3.39	
1	66	61.74	36.81	75.75	66.58	21.31	2.20	
2	76	61.67	33.82	94.07	76.38	24.44	2.53	
3	59	67.53	41.41	100.59	59.21	18.95	1.96	
4	96	39.26	29.13	48.00	96.14	30.76	3.18	
5	62	68.48	46.77	97.19	62.99	20.16	2.08	
6	48	68.45	42.70	89.53	48.70	15.58	1.61	
7	43	44.96	20.00	92.23	43.47	13.91	1.44	2.08
8	90	55.35	34.00	81.90	90.76	29.04	3.00	
9	46	71.13	40.00	97.68	46.11	14.75	1.53	
10	46	66.91	38.73	108.40	46.73	14.95	1.55	
11	59	70.45	40.00	87.19	59.68	19.10	1.97	
12	57	65.12	43.00	87.45	57.36	18.36	1.90	
1	93	54.84	37.16	68.64	93.08	29.79	3.08	3.08
1	211	99.42	68.34	174.00	211.47	67.67	7.00	
2	82	103.17	69.01	123.71	82.01	26.24	2.71	
3	158	71.94	62.00	77.63	158.06	50.58	5.23	
4	63	111.85	65.94	160.76	63.65	20.37	2.11	2.46
5	73	98.41	57.65	135.75	73.68	23.58	2.44	
6	55	108.72	59.44	155.43	55.44	17.74	1.83	
7	67	103.65	71.41	126.58	67.08	21.47	2.22	

8	50	130.59	63.00	165.85	50.54	16.17	1.67	
9	64	98.55	57.03	129.00	64.41	20.61	2.13	
10	60	114.32	68.00	156.64	60.21	19.27	1.99	
11	71	84.56	52.41	115.83	71.70	22.94	2.37	
12	81	102.07	64.00	120.04	81.86	26.20	2.71	
13	45	122.89	71.00	159.00	45.39	14.52	1.50	
14	52	108.36	53.00	167.12	52.58	16.82	1.74	
15	43	113.37	62.00	155.52	43.97	14.07	1.45	
16	51	121.71	62.00	157.86	51.67	16.53	1.71	
17	54	139.49	75.00	193.55	54.33	17.39	1.80	
18	52	108.18	66.00	160.08	52.48	16.79	1.74	
1	60	120.65	66.60	166.50	60.77	19.45	2.01	
2	223	71.54	61.00	120.01	223.53	71.53	7.40	
3	60	110.73	62.94	142.70	60.72	19.43	2.01	
4	80	134.74	92.78	243.76	80.05	25.61	2.65	
5	29	134.30	62.00	193.28	29.91	9.57	0.99	
6	57	114.72	70.00	142.54	57.84	18.51	1.91	
7	89	73.10	60.12	85.69	89.34	28.59	2.96	
8	56	111.99	77.00	158.94	56.75	18.16	1.88	
9	68	89.97	62.78	125.09	68.46	21.91	2.27	2.34
10	53	99.11	44.72	154.50	53.34	17.07	1.76	
11	48	120.06	66.47	150.34	48.53	15.53	1.61	
12	53	120.31	58.00	160.75	53.80	17.22	1.78	
13	93	72.47	59.00	89.45	93.03	29.77	3.08	
14	50	120.24	67.00	161.58	50.61	16.19	1.67	
15	74	76.94	63.25	88.89	74.25	23.76	2.46	
16	50	123.53	61.87	200.00	50.54	16.17	1.67	
17	50	82.55	31.00	161.66	50.52	16.16	1.67	
1	178	70.90	59.00	86.97	178.90	57.25	5.92	
2	61	105.61	73.25	132.39	61.86	19.80	2.05	
3	94	96.62	62.39	123.06	94.05	30.10	3.11	3.11
4	81	87.93	44.52	133.95	81.14	25.97	2.69	
5	54	78.56	66.25	98.00	54.42	17.42	1.80	

Path Length Measurements - BG8 in no calcium

	Area	Mean	Min	Max	Length	length microns	velocity (microns/s)	average velocity/group
1	96	88.46	60.15	113.23	96.23	30.79	3.18	3.18
1	99	61.49	57.00	70.91	99.44	31.82	3.29	
2	83	67.88	62.00	73.49	83.17	26.62	2.75	2.89
3	54	100.74	82.23	122.71	54.90	17.57	1.82	
4	111	72.07	64.53	78.84	111.35	35.63	3.68	
1	101	88.41	79.98	97.99	101.53	32.49	3.36	3.36
1	57	92.37	62.00	117.55	57.41	18.37	1.90	1.90
1	200	64.94	50.24	77.44	200.45	64.14	6.63	6.63

Path Length Measurements - BG8 in M media

	Area	Mean	Min	Max	Length	length microns	velocity	average velocity/group
1	142	60.88	40.59	76.71	142.51	45.60	4.72	5.83

2	190	62.90	52.57	75.55	190.22	60.87	6.29	
3	148	71.21	60.00	83.87	148.12	47.40	4.90	
4	150	61.33	40.00	93.24	150.57	48.18	4.98	
5	227	61.16	53.43	69.78	227.81	72.90	7.54	
6	236	55.44	41.31	82.32	236.34	75.63	7.82	
7	137	75.43	57.00	97.04	137.83	44.10	4.56	
1	166	83.00	55.39	230.19	166.62	53.32	5.51	
2	93	66.93	57.49	75.10	93.91	30.05	3.11	3.36
3	100	88.69	72.60	104.50	100.29	32.09	3.32	
4	45	102.61	80.00	119.05	45.35	14.51	1.50	
1	127	63.13	56.95	71.73	127.66	40.85	4.22	
2	103	80.82	62.00	92.79	103.74	33.20	3.43	
3	86	84.21	65.69	100.78	86.62	27.72	2.87	
4	242	64.69	55.15	90.57	242.75	77.68	8.03	
5	226	66.23	57.58	75.70	226.03	72.33	7.48	5.50
6	92	82.58	70.88	93.15	92.46	29.59	3.06	
7	112	80.56	63.32	99.63	112.38	35.96	3.72	
8	178	62.86	55.60	71.56	178.29	57.05	5.90	
9	288	65.62	54.29	89.41	288.61	92.35	9.55	
10	202	66.61	57.37	76.41	202.81	64.90	6.71	
1	111	88.86	63.05	135.24	111.48	35.67	3.69	
2	36	73.31	62.84	86.06	36.90	11.81	1.22	2.19
3	50	114.55	80.00	146.47	50.61	16.20	1.67	
1	137	67.77	55.00	89.15	137.68	44.06	4.56	
2	39	96.30	69.00	123.44	39.64	12.69	1.31	
3	47	106.45	59.00	135.73	47.42	15.17	1.57	10.12
4	46	93.20	67.74	114.16	46.77	14.97	1.55	
5	34	121.63	71.02	163.12	34.22	10.95	1.13	
1	56	118.27	72.91	155.31	56.71	18.15	1.88	
2	80	114.78	76.75	147.52	80.37	25.72	2.66	
3	93	69.67	51.00	91.89	93.03	29.77	3.08	
4	121	80.11	35.59	199.95	121.05	38.74	4.01	
5	71	87.05	67.06	120.80	71.28	22.81	2.36	2.37
6	30	89.45	74.00	99.43	30.81	9.86	1.02	
7	64	78.44	71.12	85.29	64.84	20.75	2.15	
8	43	87.28	72.47	101.96	43.47	13.91	1.44	
9	84	80.62	73.00	95.89	84.31	26.98	2.79	

C. Wild-type Path Length Measurements

Path Length Measurements - cc124 in calcium

	Area	Mean	Min	Max	Length	length microns	velocity	average velocity/group
1	704	59.03	35.02	69.76	704.34	225.39	23.31	
2	359	66.69	59.75	73.86	359.38	115.00	11.89	
3	224	62.68	57.00	69.97	224.32	71.78	7.42	14.41
4	715	60.82	54.19	70.00	715.71	229.03	23.68	
5	173	60.91	49.27	68.49	173.75	55.60	5.75	
1	578	63.94	57.89	69.00	578.19	185.02	19.13	
2	769	59.66	51.00	73.12	769.52	246.25	25.46	
3	709	57.31	31.19	66.27	709.14	226.93	23.47	17.11
4	210	62.75	58.14	70.43	210.20	67.26	6.96	
5	317	59.45	30.00	68.90	317.96	101.75	10.52	
1	909	60.10	52.24	71.27	909.08	290.91	30.08	
2	401	57.30	51.46	64.53	401.72	128.55	13.29	
3	163	70.22	59.00	88.59	163.33	52.26	5.40	
4	700	61.30	52.73	76.18	700.56	224.18	23.18	
5	361	60.20	54.46	67.82	361.99	115.84	11.98	17.90
6	481	62.53	38.31	91.10	482.00	154.24	15.95	
7	481	58.24	38.60	67.68	481.53	154.09	15.93	
8	910	59.52	49.38	70.94	910.44	291.34	30.13	
9	457	67.31	60.00	86.48	457.46	146.39	15.14	
1	855	60.00	51.09	78.30	855.70	273.82	28.32	
2	626	59.53	43.00	68.38	626.60	200.51	20.74	
3	420	66.13	55.00	82.62	420.53	134.57	13.92	
4	486	61.68	54.00	78.08	486.83	155.78	16.11	
5	696	62.58	56.04	71.22	696.97	223.03	23.06	
6	706	63.94	57.96	78.55	706.45	226.06	23.38	
7	722	62.42	55.00	70.00	722.17	231.10	23.90	20.31
8	701	61.93	40.19	70.88	701.57	224.50	23.22	
9	408	64.20	53.88	73.50	408.88	130.84	13.53	
10	709	61.55	55.00	69.00	709.84	227.15	23.49	
11	644	63.51	56.01	74.46	644.09	206.11	21.31	
12	257	57.70	30.00	67.89	257.42	82.37	8.52	
13	740	60.74	48.01	67.33	740.99	237.12	24.52	
1	386	61.48	56.61	68.84	386.74	123.76	12.80	
2	461	65.30	58.25	80.99	461.91	147.81	15.29	
3	519	63.58	58.02	70.80	519.11	166.11	17.18	
4	576	68.36	56.22	186.91	576.84	184.59	19.09	
5	435	61.77	51.44	128.35	435.61	139.39	14.42	
6	412	65.43	60.55	74.43	412.57	132.02	13.65	16.31
7	619	60.92	53.00	69.75	619.34	198.19	20.50	
8	447	64.06	45.76	222.92	447.39	143.17	14.81	
9	600	61.56	55.00	70.00	600.24	192.08	19.86	
10	423	64.07	51.47	71.39	423.07	135.38	14.00	
11	539	65.01	56.08	76.90	539.78	172.73	17.86	

1	838	60.43	52.23	70.17	838.70	268.38	27.75	
2	385	58.70	25.97	66.78	385.99	123.52	12.77	
3	466	54.58	24.15	66.77	466.62	149.32	15.44	
4	445	61.69	46.97	75.84	445.41	142.53	14.74	
5	302	59.39	55.11	65.91	302.36	96.76	10.01	
6	743	62.31	52.00	74.59	743.97	238.07	24.62	
7	790	59.99	53.84	68.74	790.56	252.98	26.16	
8	855	59.52	51.56	69.08	855.22	273.67	28.30	
9	353	71.24	58.00	90.53	353.13	113.00	11.69	17.21
10	526	59.52	50.38	71.52	526.97	168.63	17.44	
11	383	64.62	57.05	76.09	383.39	122.68	12.69	
12	702	64.14	57.06	73.39	702.55	224.82	23.25	
13	569	63.23	56.46	75.00	569.08	182.11	18.83	
14	277	59.64	50.47	75.64	277.69	88.86	9.19	
15	595	58.17	43.88	69.92	595.62	190.60	19.71	
16	561	63.66	55.62	73.86	561.74	179.76	18.59	
17	277	64.62	58.13	78.69	277.21	88.71	9.17	
18	282	62.37	45.00	132.55	282.54	90.41	9.35	
1	677	63.59	56.06	70.94	678.00	216.96	22.44	
2	308	53.59	22.23	64.00	308.87	98.84	10.22	
3	389	62.90	55.54	69.17	389.91	124.77	12.90	
4	162	67.66	58.21	77.86	162.29	51.93	5.37	
5	378	54.95	37.00	63.95	378.84	121.23	12.54	
6	773	61.38	55.66	69.62	773.72	247.59	25.60	16.40
7	147	62.41	51.75	72.27	147.35	47.15	4.88	
8	460	60.29	54.06	71.85	460.19	147.26	15.23	
9	720	63.15	56.33	70.19	720.54	230.57	23.84	
10	686	60.22	54.49	68.38	686.51	219.68	22.72	
11	465	61.38	23.00	71.80	465.32	148.90	15.40	
12	775	60.67	54.78	67.90	775.13	248.04	25.65	

Path Length Measurements - cc124 in M media

	Area	Mean	Min	Max	Length	length in microns	velocity	average velocity
1	308	55.54	42.80	66.96	308.02	98.57	10.19	
2	301	50.25	35.41	59.56	301.38	96.44	9.97	
3	349	50.85	42.04	59.78	349.42	111.81	11.56	
4	682	52.73	41.14	94.50	682.67	218.45	22.59	
5	788	46.79	36.16	64.06	788.72	252.39	26.10	15.02
6	480	49.90	39.20	65.15	480.41	153.73	15.90	
7	340	42.83	3.31	59.15	340.10	108.83	11.25	
8	463	53.10	43.54	66.85	463.32	148.26	15.33	
9	370	45.32	25.43	62.81	370.97	118.71	12.28	
10	496	43.69	31.00	99.40	496.22	158.79	16.42	
11	628	49.05	38.15	58.82	628.83	201.23	20.81	
12	358	52.03	40.00	67.00	358.78	114.81	11.87	17.01
13	351	58.23	41.03	123.73	351.94	112.62	11.65	
14	347	54.23	40.45	76.54	347.48	111.19	11.50	

15	900	46.81	33.25	62.23	900.19	288.06	29.79	
16	808	46.67	32.00	77.58	808.03	258.57	26.74	
17	746	51.49	38.64	87.49	746.34	238.83	24.70	
18	832	44.39	28.98	62.29	832.72	266.47	27.56	21.62
19	642	49.64	38.03	73.67	642.74	205.68	21.27	
20	378	45.70	30.58	61.09	378.44	121.10	12.52	
21	512	41.07	30.21	52.18	512.22	163.91	16.95	
1	733	56.43	31.04	65.37	733.18	234.62	24.26	
2	883	60.77	39.98	67.97	883.33	282.66	29.23	
3	578	62.39	55.30	78.43	578.77	185.21	19.15	
4	530	62.15	55.98	72.95	530.92	169.89	17.57	21.48
5	687	61.86	52.47	82.14	687.55	220.02	22.75	
6	391	55.71	45.00	69.37	391.29	125.21	12.95	
7	738	56.63	28.58	67.90	738.30	236.25	24.43	
8	692	57.85	45.01	67.72	692.16	221.49	22.90	
9	394	54.58	34.00	63.35	394.46	126.23	13.05	
10	574	62.08	54.98	76.07	574.26	183.76	19.00	
11	503	57.70	45.60	65.16	503.36	161.08	16.66	
12	327	67.41	56.01	96.80	327.66	104.85	10.84	18.16
13	724	61.25	56.47	68.81	724.97	231.99	23.99	
14	569	64.64	58.24	76.00	569.20	182.14	18.84	
15	453	59.90	39.17	81.50	453.18	145.02	15.00	
16	790	61.94	55.65	69.25	790.70	253.02	26.17	
17	459	58.35	22.00	69.49	459.23	146.95	15.20	
18	1048	59.19	26.97	69.38	1048.21	335.43	34.69	
19	447	52.14	22.00	64.09	447.94	143.34	14.82	
20	387	66.23	58.00	72.98	387.68	124.06	12.83	
21	669	62.14	57.69	71.96	669.61	214.27	22.16	
22	484	60.67	46.00	71.17	484.56	155.06	16.03	21.34
23	422	65.23	40.45	87.44	422.88	135.32	13.99	
24	757	61.41	56.00	69.79	757.67	242.45	25.07	
25	745	62.58	55.26	71.01	745.39	238.53	24.67	
26	840	61.83	56.00	74.74	840.31	268.90	27.81	